ENDOCRINOLOGY

THE BULLETIN OF
THE ASSOCIATION FOR THE STUDY
OF INTERNAL SECRETIONS

VOLUME 21 MARCH, 1937 NUMBER 2

STUDIES ON ADAPTATION

HANS SELYE

From the Department of Biochemistry, McGill University
MONTREAL, CANADA

In a preliminary publication (1) attention has been called to a syndrome which appears when a severe injury is inflicted upon the organism. This syndrome is independent of the nature of the damaging agent and represents rather a response to damage as such. Exposure to cold, traumatic injuries, excessive muscular exercise, spinal shock, acute infections, and intoxications with various drugs will evoke this syndrome if they damage the organism sufficiently. The course of this reaction, which we have interpreted as an expression of general defence, may be divided into three stages. During the first, or acute stage, observed in the rat ordinarily 6 to 48 hours after the initial injury, one notes a rapid decrease in the size of the thymus, spleen, lymph glands and liver; disappearance of fat tissue; edema formation, especially in the thymus and loose retroperitoneal connective tissue; accumulation of pleural and peritoneal transudate; loss of muscular tone; fall of body temperature; formation of acute erosions in the digestive tract, particularly in the stomach, small intestine and appendix; loss of cortical lipoids and chromaffin substance from the adrenals; and sometimes hyperemia of the skin, exophthalmos, increased lachrymation and salivation. In very severe cases, focal necrosis of the liver and dense clouding of the crystalline lens may be observed.

After a few days, however, a certain resistance is built up against the damaging stimulus. Thus, for instance, in the experiments in which such a syndrome was produced by means of a drug, we found that even though we continued to inject the animals daily with the same dose of the substance which originally evoked all these symptoms, the organ changes gradually disappeared, and finally the animals became resistant. If daily injections were continued still longer the animals lost their resistance, and in a third stage died with organ changes similar to those seen in the first stage. We have termed this reaction the 'general adaptation reaction' and its 3 stages the 'stage of alarm', the 'stage of resistance', and the 'stage of exhaustion'. The name 'stage of alarm' has been chosen for the acute stage because we consider

it an expression of the general alarm of the organism when first confronted with a damaging agent. Stimuli capable of evoking such a reaction of alarm will, for the sake of brevity, be referred to as 'alarming stimuli', and the dose of a drug or stimulus required to cause a typical alarm reaction will be called the 'alarming dose' of such a drug or stimulus. It is evident that in the case of injuries so severe that adaptation cannot occur, the alarm stage will be the only one to develop. In this case it is hardly possible to speak of an 'alarm stage', and the term 'alarm reaction' will therefore be used whenever we discuss this stage as a separate entity.[1] It must be borne in mind, however, that the alarm reaction may also represent the first stage of the adaptation reaction. The ability of an animal to offer resistance to damaging stimuli—that is, the ability to adapt itself—will be referred to as the 'adaptation energy'. This, as we shall see, is limited in quantity, and is drawn upon during the process of adaptation.

It seems difficult at first to understand why the organism should react to entirely different stimuli in an identical manner. The great similarity between the symptoms of the alarm reaction and those of histamine toxicosis or anaphylactic and surgical shock make it likely that the liberation of large quantities of histamine or pharmacologically similar substances (adenylic acid derivatives, acetylocholine, kallikrein) from the tissues plays an important part in producing this syndrome (2). The difference between the response to histamine and that to other drugs is that the former produces the characteristic symptoms at doses especially far from lethal and more regularly and promptly than any other drug studied. The latent period in the case of other drugs might represent the time necessary for the liberation of histamine from tissues. Further evidence in support of this hypothesis will be discussed in connection with our experiments in the following pages. The fact that histamine is a physiological constituent of tissues has been generally recognized since the classical demonstration of its presence in the spleen by Dale and his school (3). That large quantities of a histamine-like substance are liberated into the blood stream during surgical shock was made very probable by the studies of Cannon (4). In spite of the contrary opinion expressed in some more recent publications, this still seems to be the most plausible explanation for the symptoms of secondary traumatic shock. It should be stressed at the beginning, however, that there is no evidence at hand which would prove that the substance or substances produced during the alarm reaction are chemically identical with, or even allied to histamine. In order to avoid coining more new names than is necessary, I shall refer to this hypothetical metabolite or group of metabolites with histamine-like pharmacological actions as 'H-substance', following herein the example of Lewis (5), who used this term to designate the histamine-like substance formed in the skin during his 'triple-response' reaction. It should be stressed, however, that the substance acting in the skin

[1] A few months ago a publication by T. Klein (Wien. Arch. f. Inn. Med. **28**: 251. 1936) appeared in which the term 'alarm reaction' is used to designate the reflex inspiration and some accompanying motor phenomena elicited by fright and other psychic stimuli. Since we have already used this term in several publications for the first stage of the adaptation reaction, it would lead to confusion if we were to change it now. One should sharply distinguish, however, between Klein's respiratory reflex and the alarm reaction in the sense in which the term is used here.

is not necessarily identical with the substance formed during the alarm reaction.

That the organ changes are not due to a direct action of the damaging agent on the various tissues, as might have been supposed in cases in which the reaction was elicited by drugs, is proved by the experiments on spinal shock, in which a relatively small lesion of the spinal cord produced changes characteristic of the alarm reaction in the various organs. The assumption of a mediator which carries the effect from the site of lesion to the various organs seems justified, therefore, even though we do not know as yet whether it is transmitted by way of the blood stream or by way of the nervous system. If the above hypothesis be correct, surgical shock would represent a special form of the alarm reaction, in which H-substance is mechanically liberated from the tissues, differing from other types of the alarm reaction only in that the latter group liberate similar substances by other means [chemical (drugs), physical (cold, x-rays, ultra-violet rays), or nervous stimuli (spinal shock)].

It would hardly be worth while to describe all the experiments in which an alarm reaction was obtained by various drugs. Suffice it to mention that an alarm reaction develops after the administration of any drug administered in sublethal doses, unless the specific pharmacological actions of the drug exert such a violent selective action on vital centers (heart, respiratory center, etc.) that death ensues as a result of this selective action before any marked general damage occurs. Among others, atropine, morphine, adrenaline, vasopressin and formaldehyde have been studied more particularly, and all these drugs produced the symptoms of the alarm reaction when given twice daily for 48 hours in sublethal doses.

ALARMING STIMULI

Groups of 10 female rats, 3 months of age, were used for each experiment in this series. They received a dose of the drug in question which had been found in preliminary experiments to be just sufficient to produce an alarm reaction; that is to say, they received the alarming dose of the drug in 4 injections within 48 hours. Four animals in each group were killed at intervals during the first 24 hours, beginning at the 8th hour after the first injection, for investigation of the acute symptoms of the alarm reaction. The remaining 6 were autopsied 48 hours after the beginning of the experiment; that is, after having received the 4 injections. The drugs used were adrenaline (0.2 cc. of a 1:1000 solution twice daily), formaldehyde (0.5 cc. of a 4 per cent solution twice daily), morphine sulphate (2 cc. of a 1 per cent solution twice daily). It would be tedious to describe in detail all autopsy observations made during the acute stage in the various groups, since the findings showed only quantitative variations and were otherwise essentially the same in each group. Edema formation in the thymus, the retroperitoneal connective tissue and the omentum, and formation of peritoneal and pleural transudates were constant findings. Occasionally hemorrhagic lung edema or hemorrhages into the urinary bladder were seen. Clouding of the crystalline lens was not observed in this series, but other experiments in which higher dosages of the same drugs were

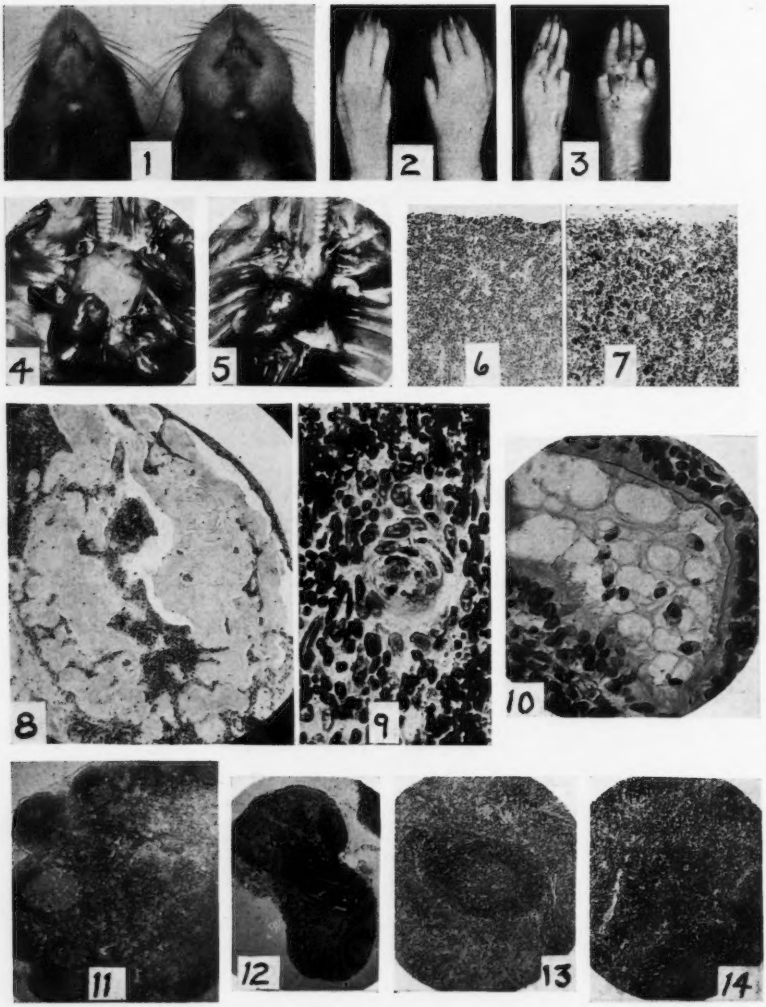

Fig. 1-14. *1, left.* Normal control rat; *right,* facial edema following injection of egg-white. *2, left.* Paw of normal control rat; *right,* edema of the paw after injection of egg-white. *3.* Same as *2,* from the plantar side. *4.* Thymus of a normal female rat in situ. *5.* Atrophic thymus of a rat in the alarm reaction (elicited by formaldehyde intoxication). *6.* Cortex of a normal rat thymus. Note the regular, normal nuclei in the thymocytes. *7.* Marked karyorrhexis and pyknosis of the thymocytes in the alarm reaction (elicited by excessive muscular exercise). *8.* Large cyst in the thymus of a rat chronically treated with estrone. Note the epithelial lining of the cyst and the dense colloid in the lumen. *9.* Giant Hassall corpuscle in the thymus of a rat in the alarm reaction (elicited by excessive muscular exercise). *10.* Cyst lined with ciliated epithelium in the thymus in the alarm reaction (elicited by surgical injury of multiple bone fractures). *11.* One of the iliac lymph glands in a normal rat. *12.* Iliac lymph glands corresponding to that shown in *11* in the alarm reaction (elicited by excessive muscular exercise). *13.* Malpighian follicle in a normal spleen. *14.* Malpighian follicle in the alarm reaction (elicited by excessive muscular exercise).

given showed that acute cataracts do develop in some cases under the influence of the non-specific damage caused by various drugs. A slight fall in body temperature (1 to 2°C.) was also characteristic during this early stage.

A summary of the most important changes in the gross weight of the

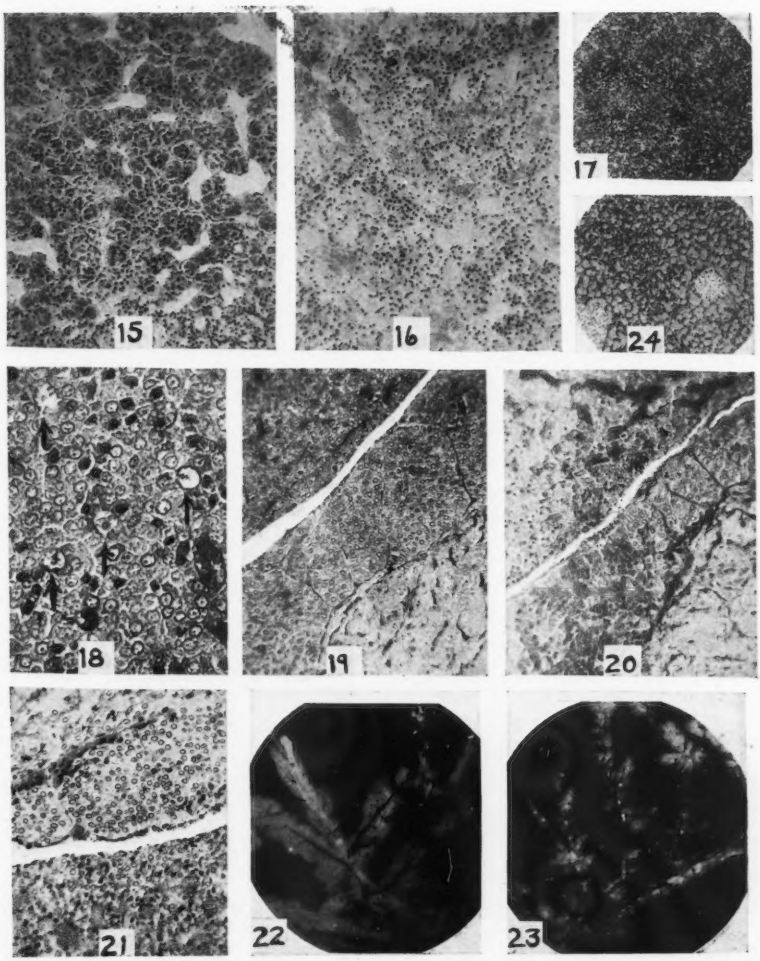

Fig. 15-24. *15.* Adrenal medulla of a normal rat. *16.* Adrenal medulla in a severe alarm reaction (elicited by excessive muscular exercise). Note the almost complete dissolution of the epithelial cells. *17.* Anterior lobe of the hypophysis in the alarm reaction (elicited by exposure to cold). Note the large number of pyknotic nuclei which appear as small black dots at this magnification. *18.* Four large 'signet-ring' cells, marked by arrows, in the alarm reaction (elicited by morphine intoxication). *19.* Hypophysis of a normal rat. Anterior lobe in lower right of the field; posterior lobe in upper left, and middle lobe between. *20.* Hypophysis in the alarm reaction (elicited by excessive muscular exercise). Note numerous necrotic cells throughout the middle lobe. *21.* Hypophysis in the alarm reaction (elicited by exposure to cold). Note the characteristic disintegration of middle lobe cells along the borderline between the middle and posterior lobes. *22.* Macroscopical photograph of the pancreas of a normal rat. *23.* Macroscopical photograph of the pancreas in the alarm reaction (elicited by 5 days' treatment with atropine). Note the distinctly visible white Langerhans' islets along the blood vessels. Pancreatic lobules are atrophic and translucent. *24.* Section through a pancreas in the alarm reaction (elicited by excessive muscular exercise). Secretory granules (extremely eosinophilic) are seen in the neighborhood of the islets; the remaining acinar tissue shows no sign of secretion.

organs 48 hours after the first injection is given in table 1. The weights given in this and following tables represent averages from the entire group. The numbers in brackets represent the smallest and the largest weight noted. Six rats, 3 months of age, have been used in each group. The weight of the iliac group of lymph glands has been recorded as a representative sample of the

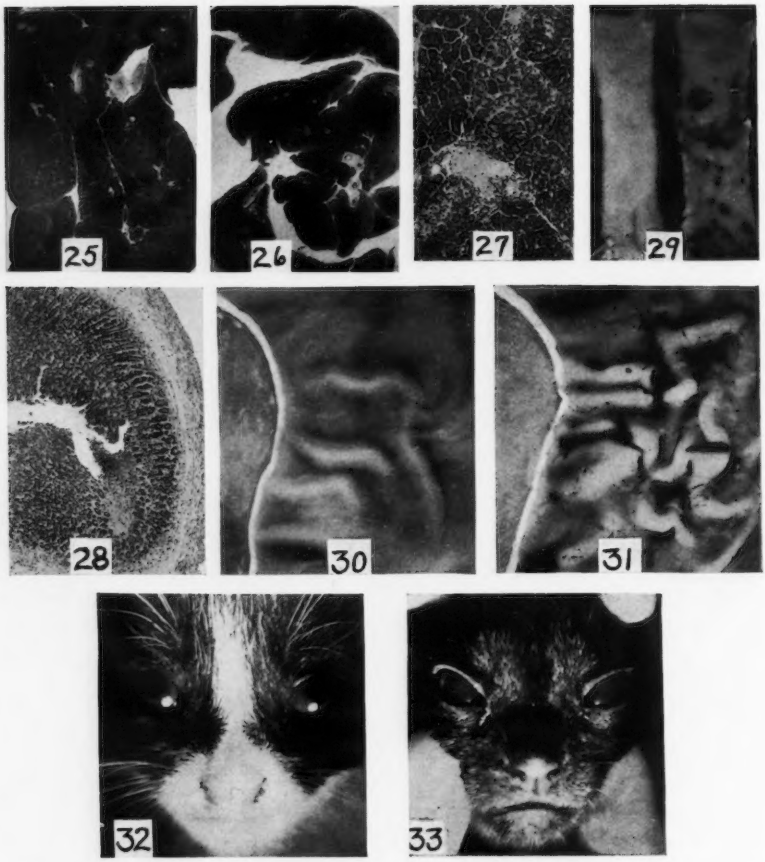

Fig. 25-33. *25.* Low magnification of pancreas of a normal rat. *26.* Pancreas in the alarm reaction (elicited by intoxication with formaldehyde), at same magnification as *25.* The Langerhans' islets are greatly enlarged and very numerous. *27.* Section of the pancreas in the alarm reaction (elicited by exposure to cold), showing cells which seem to represent intermediate stages between acinar and islet cells. *28.* Ulcer covered by a slough of necrotic material in the depth of a fold in the gastric mucosa of a rat in the alarm reaction (elicited by formaldehyde intoxication). *29.* Macroscopical photographs of the mucosa of the small intestine; left, normal mucosa; right, mucosa covered with deep ulcers in the alarm reaction (elicited by exposure to cold). *30.* Macroscopical photograph of the gastric mucosa of a normal rat; left, the proventriculus; right, the secretory epithelium of the ventriculus. *31.* Deep hemorrhagic ulcers in the gastric mucosa in the alarm reaction (elicited by exposure to cold). *32.* Eyes of a normal cat. *33.* Cataract formation in the cat in the alarm reaction (elicited by exposure to cold).

lymphatic system. This group consists of 4 nodes, 1 on each side of the inferior vena cava above its bifurcation into the 2 iliac veins, 1 just below the bifurcation between the 2 iliac veins, and 1 in the mesentery of the rectum just ventrally to the last-mentioned node.

From table 1 it is obvious that after 48 hours—that is, at the end of the first, or alarm stage of the adaptation reaction—the most striking changes are the sudden involution of the thymus, and less regularly that of the lymph glands and spleen; furthermore, the marked hypertrophy of the adrenals. The histological changes in these organs will be described later, together with microscopical changes in other organs not accompanied by marked variations

in total weight. It will suffice here to discuss those most outstanding features of the alarm reaction which make the diagnosis of the syndrome possible, and thus allow one to discover the conditions which will elicit an alarm reaction. In addition to the changes in the weight of the lymphatic organs and the adrenals, a characteristic symptom is the formation of gastric and intestinal erosions which, although not constant, are frequently observed during the acute stage. They do represent an essential symptom, however, and are invariably observed if adrenalectomized animals are exposed to alarming stimuli. The effect of adrenalectomy on the development of these gastro-intestinal changes will be discussed later. The water retention and edema formation so characteristic during the first 24 hours of the alarm reaction subside at the end of this stage and are only rarely observed after 48 hours.

TABLE 1. CHANGES IN THE WEIGHT OF THE LYMPHATIC ORGANS AND THE ADRENALS IN THE ALARM REACTION. AVERAGE, SMALLEST AND LARGEST WEIGHTS, IN MG.

	Thymus	Lymph Glands	Spleen	Adrenals
Untreated	*258*, (172, 400)	*38*, (19, 72)	*850*, (440, 1,400)	*53*, (44, 62)
Adrenaline	*94*, (70, 114)	*26*, (22, 34)	*520*, (300, 800)	*63*, (56, 80)
Formaldehyde	*85*, (72, 110)	*15*, (10, 29)	*370*, (200, 540)	*67*, (54, 75)
Morphine	*114*, (97, 195)	*36*, (26, 46)	*740*, (370, 900)	*67*, (62, 78)
Atropine	*124*, (72, 285)	*32*, (19, 38)	*810*, (750, 870)	*80*, (60, 100)
Spinal Shock	*114*, (64, 230)[1]	*18*, (12, 24)	*560*, (520, 630)	*63*, (58, 68)
Surgical Shock	*88*, (51, 170)	*26*, (18, 34)	*570*, (510, 640)	*61*, (40, 70)
Cold	*137*, (81, 187)	*24*, (18, 27)	*540*, (240, 700)	*62*, (54, 80)
Exercise	*124*, (77, 215)	*34*, (23, 62)	*620*, (380, 900)	*62*, (48, 74)

[1] Since the thymus edema had not quite subsided within 48 hours in this group the weights do not give an accurate image of the amount of thymus tissue left at this time.

As mentioned above, not only drugs but many other damaging stimuli may evoke an alarm reaction; the following examples illustrate the production of this syndrome by surgical shock, spinal shock, exposure to cold, and excessive muscular exercise. In every one of these cases, groups of 10 female rats, 3 months of age, were used, 4 of which were killed during the first 8 to 24 hours, and showed symptoms identical with those seen after the administration of alarming doses of various drugs. The only noteworthy difference was the higher incidence of gastro-intestinal ulcers in the group in which the alarm reaction was elicited by means other than drugs. Surgical shock was produced under ether anesthesia by exposure of the intestines for a period of 1.5 minutes, during which time the alimentary tract, the spleen, and omentum were exteriorized and slightly handled. Spinal shock was elicited by transverse section of the spinal cord just below the level of the vertebra prominens. The group exposed to cold was placed in an icebox at a temperature of —6°C. during the entire period of the experiment, 48 hours. The animals in which the effect of excessive muscular exercise was studied were placed in revolving cages having a diameter of 12 inches, which were driven by an electric motor at a speed of 18 to 22 revolutions per minute, for 1.5 hours twice daily. The weight changes observed in the lymphatic system and in the adrenals after 48 hours are summarized in table 1.

From this it is obvious that the changes caused by these various procedures are identical with those observed after the administration of alarming doses

of various drugs. The increase in adrenal weight and decrease in thymus weight are especially characteristic and constant. The atrophy of the lymph glands and the spleen, though always detectable by microscopical investigation, is not so apparent when judged by the gross weight.

It is concluded that the alarm reaction really represents a response to damage as such and not to any specific action of the drugs or other damaging agents tested. One would be tempted to interpret the well-known fact that extensive skin burns often cause gastro-intestinal erosions and concomitant adrenal changes in humans as an expression of the alarm reaction. In this connection it is of interest to mention the work of Horton, Brown and Roth (6), who found in the course of observations on human subjects, that immersion of the extremities in cold water will increase gastric secretion, decrease the blood pressure and cause marked generalized hyperemia of the skin, particularly in certain predisposed individuals. They interpret this response as being due to histamine liberation. In connection with our work on the effect of excessive muscular exercise, the observations of Andersen (7) are of interest. This author found slight decrease in thymus size without histological changes, and an increase in the lipoid content of the adrenals, in rats after exercise. The reason for her failure to observe the histological characteristics of accidental thymus involution and the loss of cortical lipoids here described, is possibly that the autopsies were not performed at the same stage of the reaction, and that the exercise used in her experiments was perhaps not as exhausting as under the conditions of our experiments. Recently, Anrep and Barsoum (8) demonstrated an increase in the histamine content of the venous blood coming from muscles during contraction. This finding seems to be in accord with the theory attributing the symptoms of the alarm reaction following excessive muscular exercise to an over-production of H-substance. In connection with the condition of spinal shock, the experiments of Ungar and his coworkers (9, 10) are of the greatest interest. They show that excitation of the peripheral stump of the purely sensory internal saphenus nerve increases the gastric secretion in the dog. This effect is not obtained if the femoral vein is ligated. The effect is not abolished by atropine. This fact, and the shape of the secretion curve indicate, according to the authors, that the active principle is probably histamine-like in nature. This would imply that H-substance is formed in peripheral tissues under the influence of nervous stimuli, and it is possible that a similar mechanism is at work in the production of the symptoms of the alarm reaction in the condition of spinal shock described above. Tinel and Ungar (11) showed, furthermore, that injection of zinc chloride and silver nitrate solutions into an artery of the dog's paw causes a sharp rise in the gastric secretion which is more marked immediately after transcision of the crural and ischiadic nerves than if the innervation of the paw is intact. It is not seen, however, after the nerves have undergone complete degeneration. This has been ascribed to the liberation of histamine-like substances from the tissues of the paw under the influence of these drugs.

This section on alarming stimuli should not be concluded without mention of the fact that parenteral administration of substances which are removed from the site of injection by phagocytosis rather than by simple absorption (such as rat blood, cholesterol emulsions, kerasin emulsions, egg-yolk and egg-white) cause an increase in the size of the lymph glands, the spleen and the liver. In this, the response to high doses of these substances differs from the usual type of the alarm reaction, even though thymus involution and adrenal hypertrophy are present just as after other alarming stimuli.

While all the substances of this series enlarged the lymph glands, the

spleen and the liver, the group treated with egg-white showed rather singular symptoms and therefore deserve a more detailed description. Twelve female rats, 3 months old, received an intraperitoneal injection of 1 cc. of pure, fresh egg-white. One to 3 hours following the injection, all of them showed marked subcutaneous edema with a characteristic distribution. The face, tongue, paws and clitoris were greatly swollen and edematous; the color of the edematous regions was bright red because of the accompanying hyperemia (see fig. 1, 2 and 3). A similar, but less pronounced edema was elicited by a second injection of 1 cc. the next day, while on the third day 6 of the animals, and on the fourth day all of them, failed to react. It appears that habituation occurred to this particular effect of the egg-white. In another experiment it was shown that this peculiar effect of egg-white (which was never observed after any other substance) may also be inhibited by giving a single injection of an alarming drug just prior to the injection of the egg-white. A group of 12 female rats, 3 months old, received 0.25 cc. of a 4 per cent formaldehyde solution subcutaneously, and immediately after this 1 cc. of egg-white intraperitoneally. Only 2 of them showed any subcutaneous edema, and even in these the reaction was delayed and very mild. That this inhibition is not simply the result of the lowered vitality of these animals was shown in a third group of 12 female rats, 3 months old, which received an injection of 1 cc. of egg-white intraperitoneally 24 hours after adrenalectomy. They all developed very marked subcutaneous edema which differed from that seen in normals only in that it was accompanied by cyanosis and not by arterial hyperemia. These animals were so severely damaged by this injection that all but 3 died within 48 hours. The significance of this peculiar reaction to egg-white is not clear, but it seems evident that in this case an alarm reaction, elicited by another drug, exerted a protective influence. As a rule the adaptation is specific, however, and numerous experiments have shown that while adaptation is acquired to a certain stimulus the animals become less resistant to other stimuli.

SYMPTOMS OF THE ALARM REACTION

One of the most striking and constant changes is the involution of the *lymphatic organs*. The loss of weight is most marked and rapid in the thymus (fig. 4 and 5). Here the characteristic cells of the parenchyma, the thymocytes, actually disintegrate and 24 hours after the onset of a severe alarm reaction only the debris of their chromatin is left (fig. 6 and 7), lying partly free in the reticulum, partly in phagocytes which are engaged in removing it. At this stage, the connective tissue stroma of the organ is usually very edematous, so much so that the entire organ assumes a gelatinous appearance which makes the naked-eye diagnosis of the condition easy. The cells of the reticulum, on the other hand, show signs of hyperplasia and by the end of 48 hours many of them assumed an epithelial appearance. They become round, their cytoplasm becomes more voluminous, and they form irregular strands of polygonal cells, tubules and even cysts, which are filled with an eosinophilic colloid similar to that seen in thyroid acini. Transitions between these new formations and typical Hassall bodies are frequently seen, and a close relationship between the two is obvious (see fig. 8, 9 and 10). The epithelial structures just de-

scribed may represent undifferentiated and hyperplastic precursors of Hassall's corpuscles. The extremely basophilic nuclear debris is usually removed by the end of 48 hours, and then only the now modified reticulum is left. This explains the great loss in total weight which the organ exhibits at this time. The lymph glands also show signs of involution, but without any noticeable hyperplasia of the reticulum. The involution in them usually begins in the germ centers, which may disappear almost completely (fig. 11 and 12). Nuclear debris is seldom found in the lymph glands and never abundantly as in the thymus, possibly because many of the lymphocytes emigrate from the lymph glands without previously undergoing necrosis, or because the drainage is better in the lymph glands than it is in the thymus, so that the debris is removed more rapidly and consequently never accumulates to any great extent in the organ. Frequently the sinuses of the lymph glands are filled with erythrocytes, so that the glands assume the appearance of hemolymph nodes. The spleen and the bone marrow usually contain but little of the nuclear debris and edema which are so characteristic of the thymus in this reaction. The atrophy of the spleen begins in the center of the Malpighian follicles and the white pulp is more severely affected than the red (fig. 13 and 14).

The *adrenal cortex* shows signs of marked hyperplasia in the alarm reaction. During the first 24 hours this is associated with a rapid loss of its lipoid granules. This loss of lipoids is apparent upon gross inspection, since the organ loses its characteristic light-yellow color and turns dark brown. In fact, this large brown adrenal, usually embedded in a translucent layer of retroperitoneal edema is probably the most typical macroscopical sign of an alarm reaction. Microscopic examination of frozen sections stained with Sudan III show that practically all Sudan-tingible granules have disappeared at this time. At the end of the alarm stage, the lipoid granules reappear, first in the shape of extremely fine, powder-like dots, more evenly distributed throughout the various layers of the cortex. Later they enlarge and localize more particularly in the zona fasciculata; that is, in that layer which contains most of these granules in normal, untreated animals. This reappearance of the lipoid granules at the end of the alarm stage was observed even if exposure to the alarming stimulus was continued, and the cortex was usually found to be loaded with lipoids during the second or resistant stage of the adaptation syndrome, irrespective of the nature of the stimulus to which adaptation occurred.

The *adrenal medulla* loses its chromaffin granules during the stage of alarm. Vacuoles appear in the periphery of its cells, and if the alarm reaction is very severe the cells may even undergo necrosis (fig. 15 and 16). In several cases this necrosis was so widespread that normal, live endocrine, or nerve cells could not be detected in the medulla of either adrenal. The stroma of the medulla and that of the adrenal cortex, however, were not markedly damaged. One cannot say, of course, whether these particular animals would have survived, and whether regeneration of the medulla would be possible in such severe cases, but animals in the resistant stage show no such signs of medullary damage, and even the chromaffin granules reappear after a certain

time. The paraganglia have not as yet been examined, but if a similar necrosis should also take place in the extra-adrenal accumulations of chromaffin tissue, such animals in the alarm reaction, or immediately after recovering from it, might prove of some use for the study of the symptoms of adrenaline deficiency. It should be mentioned in this connection that, apart from the extreme muscular weakness, the general condition of the animals in which the necrosis of the medulla was complete did not differ appreciably at the time they were killed from that of other rats in the same group which recovered.

The *hypophysis* also shows very characteristic changes in all 3 lobes. In the anterior lobe very numerous pyknoses are invariably found during the acute stage. These pyknoses appear in patches in certain regions of the gland, while other parts remain relatively free (see fig. 17). After more chronic treatment with non-specific damaging agents, an increase in the number of basophiles is frequently observed, and many of these basophiles assume the 'signet-ring cell' appearance. They are not unlike castration cells (see fig. 18). The changes in the middle lobe are most obvious during the stage of alarm. They consist in degeneration of the epithelial cells, especially along the junction line between the middle and the posterior lobe (fig. 19, 20 and 21). The debris of such degenerated middle lobe cells invades the posterior lobe. Many degenerated and even relatively unchanged middle lobe cells are found at this time in the tissue of the posterior lobe. The changes in this part of the gland consist mainly of vacuolization of the posterior lobe tissue and infiltration with more or less degenerated debris of middle lobe cells. The vacuolization is most marked in the vicinity of blood vessels and is probably due to edema. In some places the degeneration of the middle lobe parenchyma goes so far that a cleft is formed in this lobe, so that the anterior lobe cells come in direct contact with the posterior lobe. A study of sections through such places may give the impression that anterior lobe cells might invade the posterior lobe through these channels directly. It is, however, difficult to decide whether this actually occurs or not. Some of the cells in the posterior lobe are filled out with vacuoles so that their cytoplasm assumes a foam-like appearance. These cells of the posterior lobe correspond to the 'Schaumzellen' of the German authors. They are normally present in the posterior lobe of senile animals. It should be pointed out that the signet-ring cells are also physiologically present in the pituitary of senile (especially male) rats.

Very marked changes in the *pancreas* are revealed even on macroscopical inspection. The organ loses its natural whitish color, becomes pink and translucent. The Langerhans' islands are readily distinguishable in the form of white spots, usually situated along the course of the larger blood vessels (see fig. 22 and 23). Histological sections show that the acinar tissue has ceased to secrete in large areas of the glands; its cells are depleted of their secretory granules. They become round and very similar to the cells of the islands of Langerhans. In the immediate vicinity of the Langerhans' islands the acinar cells retain their secretory granules much longer than in regions farther away from them (fig. 24). The granules here are very eosinophilic and a halo of eosinophilic cells surrounding the Langerhans' islands is one of the characteristic physiological changes in the pancreas during the alarm reaction.

The Langerhans' islands themselves are usually enlarged, especially in the later stages, and many new islands are formed (fig. 25 and 26). These new islands seem to bud out from pancreatic ducts. Sometimes, however, one gains the impression that acinar cells undergo a direct transformation into island cells. One may find all intermediate stages between the typical acinar cell which rounds off after having lost its secretion granules and the characteristic Langerhans' islands cells. The question whether such direct transformation of acinar cells into endocrine cells is possible has not been decided as yet; while Opie (12) states that island cells may only be formed from ducts, but never from acini, Herxheimer (13) maintains that transitions are possible, and that island cells may be formed from acini and vice versa. Sections such as that represented in figure 27 certainly give the impression that islands do form from acinar tissue. However this may be, the fact remains that the enlargement of the Langerhans' island is not the result of any mitotic division of the island cells themselves, since in all our material we have seen only 2 cases of Langerhans' cells actually in the process of mitosis. The fact that the islands become so readily visible to the naked eye during the alarm reaction cannot be ascribed solely to their enlargement, however, but is due first of all to the fact that the acinar tissue assumes a translucent pinkish color after the secretory granules are lost, and thus the white islands become much more obvious.

The *gastric and intestinal ulcers* appeared histologically to be due to complete dissolution of the mucosa and are often accompanied by inflammatory infiltrations in the submucosa and muscularis (fig. 28, 29, 30 and 31). Perforating gastric ulcers have repeatedly been observed; they led to abscess formation in the peritoneal cavity in the immediate vicinity of the perforation. Generalized peritonitis never ensued, since adhesions of the omentum and intestines demarcated the inflammatory process.

It is hardly within the scope of this communication to discuss fully the literature concerning the production of gastric ulcers by various experimental procedures. We feel, however, that it would be of some value at this time to correlate our findings with some of the more significant observations which other investigators have made in this field. It will be recalled that such ulcers have been produced by a great variety of drugs, injuries to the vegetative centers in the hypothalamic region, skin burns, etc. A publication by Sun (14) is of particular interest in connection with the present observations. This author, studying the effect of starvation, found that the tips of intestinal villi disintegrate after 24 hours' starvation in the rat if the animals are kept at a surrounding temperature of 10°C., while in warmer surroundings longer starvation is necessary to produce the same result. The interesting observations made by Dodds and Noble (15) that extracts of the posterior lobe of the pituitary produce ulcers in the gastric mucosa of various animals may also have some relation to this problem. It is difficult to understand why such a variety of stimuli should act in an identical fashion. The theory that H-substance is liberated from the various tissues during the alarm reaction may give a plausible explanation of these observations. It is indeed well known that histamine causes marked erosions of the gastric mucosa in various animals (16, 17, 18, 19, 20, 21). These erosions are especially easily produced after adrenalectomy (2), while adrenalectomy in itself does not cause gastric erosions in the rat.

In connection with the production of gastric ulcers by hypothalamic in-

jury, it may be recalled that the experiments described earlier in this paper showed that transcision of the spinal cord also produces acute gastric and intestinal erosions. It seems that at least in the rat the lesion does not necessarily have to be in the hypothalamus, but that a condition of spinal shock exerts a similar effect. This does not mean, however, that an injury to the hypothalamic centers is not more likely to cause gastro-intestinal erosions than a lesion in any other part of the central nervous system, but it is very likely that erosions produced by hypothalamic injuries are only one of the symptoms of an alarm reaction caused by interference with the important centers of this region.

The effect of extracts of the posterior lobe of the hypophysis deserves special consideration. It would not be justifiable to consider the ulcers produced by vasopressin simply as a result of the nonspecific damage caused by high doses of this drug. Indeed, we found that the dose of vasopressin necessary to produce a gastric erosion is relatively small in proportion to its general toxic action. In this respect the effect of vasopressin, histamine, section of the spinal cord, excessive muscular exercise and exposure to cold differs from that of numerous drugs in that the former group invariably produce marked gastric erosions in the normal rat. In the case of drugs other than histamine or vasopressin, even lethal doses have little effect, if any, on the gastric mucosa of the normal animal, while marked erosions are produced by any one of them after adrenalectomy. These apparent discrepancies may be explained by the assumption that the specific toxicological action of the drug causes death before the organism was able to liberate enough H-substance to produce gastric erosions. In the case of exposure to cold, excessive muscular exercise or spinal shock, a form of intoxication with histamine-like metabolites is produced, uncomplicated by the toxicological effects of any exogenous pharmacological agent. Summarizing, it seems that the most plausible explanation of the gastric ulcers experimentally produced by various agents is the following. Various drugs and other damaging agents liberate H-substance from the tissues, which exerts a toxic effect on the mucosa of the stomach and intestines. This H-substance is detoxified by the adrenal or produced in unusually large quantities in the absence of this gland, so that ulcers are more readily obtained after adrenalectomy.

The *liver* also undergoes a marked loss of weight during the alarm reaction and occasionally shows fatty degeneration (demonstrated by staining frozen sections with Sudan III) or cloudy swelling. In rare instances focal necroses may form.

Cloudy swelling in the *kidneys* has also been observed. The urine is often stained with blood during the alarm reaction. Bladder hemorrhages have also been seen, but only in very severe cases.

Clouding of the *crystalline lens* was found only in especially severe cases. The clouding is transitory in nature and disappears if the animals recover from the alarm reaction. It has been observed in rats treated with various drugs or exposed to cold, and it was particularly common in a group of animals in which an alarm reaction had been elicited by tying their legs together for 48 hours, which led to excessive struggling during this period. The same

acute development of cataracts has been observed in 4 cats, 4 weeks of age, 3 hours after having been placed into a surrounding temperature of —1°C. As soon as they were warmed up again, the clouding disappeared. It is noteworthy that the formation of cataracts and of gastro-intestinal ulcers is especially common if the body temperature falls markedly below normal during the alarm reaction (see fig. 31 and 32).

STIMULI INFLUENCING THE COURSE OF THE ADAPTATION REACTION

The course of the adaptation reaction is very readily influenced by the conditions of the surroundings and even by the past history of the animals—that is, by those stimuli to which they have had to adapt themselves previously. One may even go so far as to say that it is not so much the dose in which a stimulus is given that makes it alarming as the condition of the animals at the moment when the stimulus is applied.

Hunger and cold are very active factors in aggravating the effects of alarming stimuli. In fact, cold or any other stimulus which is able to cause an alarm reaction by itself acts in a similar way. Thus it is possible, for instance, to obtain a typical alarm reaction by giving one quarter of the alarming dose of 4 different drugs within 48 hours, although one quarter of the alarming dose of any one of these drugs has no effect by itself.

The age and sex of the animals is also of importance. Old animals react more readily than young ones, possibly because much of their adaptation energy has already been used to meet the incidental demands of life. This probably explains also why resistance against almost any alarming stimulus is more difficult to obtain in fully adult rats, a fact which led to the use of animals 2 to 3 months of age in preference to older ones for the purposes of the present experimental series. Females show a more marked involution of the lymphatic organs after exposure to alarming stimuli than males. This may find its explanation in the fact that the adrenals are larger in the female rat than they are in the male.

The fact that hypophysectomy lessens the accidental involution of the thymus, and that adrenalectomy inhibits it completely, has been reported in a previous communication (22). So far, the only substances with which we have been able to cause thymus involution in the adrenalectomized rat are cortical extract and estrone (23). While adrenalectomy and, to a lesser extent, hypophysectomy, inhibit thymus involution, both of these operations facilitate the production of the other symptoms of the alarm reaction, such as the gastro-intestinal ulcers, the edema, the decrease in body temperature, and so forth. That hypophysectomy likewise inhibits the enlargement of the adrenal under the influence of alarming stimuli has also been noted (24).

Our recent experiments show that the lymph glands and the spleen, like the thymus, decrease in weight during the alarm reaction in the normal animal but not in the adrenalectomized rat. Table 2 summarizes a series of experiments which were performed on 3 months-old rats, using 10 males and 10 females in each group. Adrenalectomy was performed 48 hours before the first injection, and 4 injections of 0.5 cc. of 4 per cent formaldehyde were given subcutaneously within 2 days. All the adrenalectomized animals in this

TABLE 2. EFFECT OF THYROIDECTOMY, ADRENALECTOMY AND GONADECTOMY ON THE ORGAN WEIGHTS IN THE ALARM REACTION. AVERAGE, SMALLEST AND LARGEST WEIGHTS, IN MG.

Conditions of Experiments	Thymus	Iliac Lymph Glands	Spleen	Adrenals	Thyroid	Hypophysis
Normal untreated ♀s	*189* (135, 235)	*25* (14, 34)	*830* (760, 1000)	*59* (48, 69)	*19* (13, 24)	*8* (7, 10)
Normal untreated ♂s	*296* (262, 368)	*42* (26, 76)	*1000* (880, 1200)	*37* (31, 41)	*22* (18, 26)	*8* (6, 10)
Normal+formaldehyde. ♀s	*118* (61, 182)	*21* (10, 32)	*560* (260, 800)	*72* (60, 90)	*18* (16, 21)	*9* (8, 10)
Normal+formaldehyde. ♂s	*123* (74, 192)	*27* (18, 42)	*650* (470, 840)	*52* (42, 66)	*20* (18, 26)	*9* (6, 10)
Thyroidectomized ♀s	*185* (112, 250)	*22* (14, 35)	*730* (480, 850)	*49* (38, 61)		*10* (8, 12)
Thyroidectomized ♂s	*209* (166, 254)	*36* (20, 56)	*780* (450, 980)	*35* (30, 40)		*9* (7, 11)
Thyroidectomized+formaldehyde. ♀s	*104* (26, 160)	*13* (8, 22)	*240* (180, 370)	*56* (50, 60)		*9* (8, 11)
Thyroidectomized+formaldehyde. ♂s	*82* (42, 107)	*21* (14, 34)	*370* (200, 600)	*47* (36, 58)		*8* (7, 10)
Adrenalectomized ♀s	*276* (158, 345)	*36* (25, 53)	*800* (640, 850)		*18* (13, 20)	*10* (7, 12)
Adrenalectomized ♂s	*293* (182, 408)	*49* (33, 58)	*1600* (810, 1500)		*20* (14, 28)	*9* (7, 10)
Adrenalectomized+formaldehyde. ♀s	*238* (147, 320)	*46* (36, 60)	*770* (700, 840)		*16* (14, 20)	*10* (8, 11)
Adrenalectomized+formaldehyde. ♂s	*322* (230, 420)	*57* (39, 89)	*1800* (900, 1500)		*18* (9, 28)	*9* (7, 10)
Adrenalectomized and thyroidectomized. ♀s	*244* (167, 340)	*35* (27, 42)	*670* (500, 1000)			*8* (7, 9)
Adr. and thyr.+formaldehyde. ♀s	*210* (165, 250)	*17* (7, 25)	*770* (500, 1300)			
Spayed females	*375* (318, 415)	*39* (31, 53)	*1100* (820, 1300)	*50* (37, 71)	*19* (14, 22)	*13* (12, 16)
Spayed females+formaldehyde	*137* (75, 290)	*36* (20, 45)	*620* (340, 800)	*60* (51, 68)	*17* (16, 18)	*13* (10, 14)
Spayed males	*412* (263, 655)	*48* (35, 72)	*1220* (800, 1700)	*48* (42, 60)	*21* (15, 28)	*15* (12, 16)
Spayed males+formaldehyde	*193* (124, 250)	*33* (22, 48)	*740* (610, 820)	*52* (48, 60)	*18* (13, 20)	*13* (11, 14)

and the following experiments received a 0.9 per cent sodium chloride solution in place of drinking water, since such treatment with salt has been found to restore the resistance of adrenal-deficient rats to various drugs without restoring the ability of thymus to undergo accidental involution (22). The figures in the table represent the averages and (in brackets) the extreme variations of the organ weights 48 hours after the first injection.

In the same table are reported the organ weights of 20 male and 20 female rats, 3 months of age, which had been thyroidectomized 2 weeks before the first injection. Ten in each group were treated with formaldehyde and 10 left as untreated controls. This experiment was performed mainly because several authors claim to have obtained thymus involution after thyroidectomy. Thus Marine and his coworkers (25), studying the effect of adrenalectomy and thyroidectomy on the thymus of the rabbit, state that thyroidectomy causes thymus involution and prevents the hyperplasia of the

thymus otherwise observed after adrenalectomy. Hence they conclude that thyroid hormone is necessary for the thymus hyperplasia which usually follows adrenalectomy.

The figures in table 2 show no significant deviation from the normal in the thymus weight of thyroidectomized but otherwise untreated rats. It appears, therefore, that thyroidectomy as such does not cause thymus involution. The thyroidectomized animals treated with formaldehyde show, however, that a dose of this drug which is not fatal for normal animals killed 4 of 10 males and 8 of 10 females in the thyroidectomized group, with very severe symptoms of the alarm reaction. Gastric and intestinal ulcers were present in all but 2 animals in this series and the involution of the lymphatic organs was much more severe than it was in the normals. It appears that thyroidectomy, although it does not lead to an alarm reaction by itself, sensitizes the organism to alarming stimuli. This would also explain the observation that rats killed 2 to 4 days after thyroidectomy almost invariably have a small thymus, probably as a result of the accompanying surgical trauma. Within 14 days, however, the thymus reassumes its normal size. The completeness of the thyroidectomy was carefully checked at autopsy in every animal of this series.

Since these experiments show that adrenalectomy inhibits and thyroidectomy facilitates the involution of the thymus under the influence of alarming stimuli, it seemed of interest to establish how the lymphatic system of animals both adrenalectomized and thyroidectomized would respond. Twenty female rats, 3 months old, were adrenalectomized and 2 days later thyroidectomized. Then, after 9 more days, 10 of these rats were treated with formaldehyde in the same way as the other animals of this series. The remaining 10 rats served as controls. The organ weights of these animals, which are also included in table 2, show no significant deviation from the normal either in the formaldehyde-treated or in the non-treated group. From this we may conclude that thyroidectomy does not influence the inhibiting action which adrenalectomy exerts on the involution of the lymphatic organs following alarming stimuli.

It seems of interest, furthermore, to establish whether castration would have any effect on the course of the alarm reaction, since the ovarian hormone estrone was found to act as an alarming stimulus and even to occupy a rather special position among the alarming stimuli inasmuch as it led to thymus involution in the absence of adrenal tissue (23).

Twenty male and 20 female rats were gonadectomized at the age of 1 month—that is, 2 months before the time at which they were to be used for the present experiment. This lapse of 2 months between the operation and the experiment was allowed in order to permit the lymphatic system to show such changes as would occur after castration. At the age of 3 months these animals were divided into 2 groups. Ten male castrates and 10 female castrates were treated with an alarming dose of formaldehyde, while the remaining 10 animals of each group served as untreated controls. The results, which are summarized in table 2 show, first of all, that the great increase in body weight seen in older animals after gonadectomy was not noticeable in these young rats; in fact, their average weight was slightly below that of normal controls. The untreated, gonadectomized animals showed a great increase in weight, not only of the thymus but of all lymphatic organs. That

this increase was even more marked than that seen in the adrenalectomized group is not significant, however, since the adrenalectomies had been performed only 4 days before the animals were sacrificed, so that the lymphatic organs did not have as much time to proliferate as they had in the gonadectomized series. The sex difference in the size of the adrenals disappeared almost completely, for the adrenal weights of the male castrates were above and those of the female castrates slightly below normal. After treatment with formaldehyde, the characteristic involution of the lymphatic organs and hypertrophy of the adrenals was well marked, in both the male and the female castrates.

In connection with the apparent hypertrophy of the lymphatic organs following gonadectomy, it should be emphasized that these organs do not show true hyperplasia in the sense of an enlargement above normal size. They only tend to assume and maintain the maximum physiological size which they would have under optimum conditions at the optimum time of their development—that is, at about 2 months of age for the thymus, and 3 to 4 months of age for the lymph glands. Objection has already been made (22) to interpreting the condition of the thymus after adrenalectomy as a state of true hyperplasia. The thymus, the lymph glands and the spleen of a gonadectomized or adrenalectomized rat are never above the maximum size that these organs may assume in the normal under optimum conditions. It is true that the average size of the lymphatic organs of a whole group of adrenalectomized or gonadectomized animals will usually be above the average size of these organs in a group of normals, even at the age of their maximum development (2 to 3 months); but this is due only to the fact that the lymphatic organs of each individual animal in the gonadectomized and adrenalectomized group were in the vicinity of the upper limit of the normal. This seems of importance because it suggests that adrenalectomy affects the lymphatic organs merely by rendering inoperative such stimuli as would be liable to cause involution, rather than by direct stimulation of their growth, as has been previously assumed. The mechanism of action of gonadectomy is different, since removal of these glands, though it maintains the lymphatic organs in a condition of maximum development, is unable to prevent their involution following alarming stimuli. Since ovarian and testicular hormones are known to cause thymus involution, it seems likely that castration acts simply by removing the source of these hormones, which would exert a constant depressive action on the lymphatic organs of postpubertal animals.

In connection with the effect of adrenalectomy on the course of the adaptation reaction, one more point deserves mention. Although adrenalectomy decreases the resistance of the rat against alarming stimuli, such adaptations as have been acquired before adrenalectomy are not entirely lost after the removal of this gland. This is well shown by the following experiment performed on 60 female rats. Twenty of these animals were pretreated with subcutaneous injections of 1 per cent morphine sulphate solution in doses ascending from 1 to 3 cc. twice daily within one month. At the end of this period, these animals were 4 months old. At this time they were adrenalectomized, together with 20 non-pretreated controls of the same age. Beginning 24 hours after this operation, these 40 adrenalectomized animals and 20 non-pretreated normal controls of the same age received 3 cc. of the morphine

solution twice daily for 3 consecutive days. While all of the non-pretreated adrenalectomized animals died during this time, only 5 of the pretreated adrenalectomized, and only 2 of the non-pretreated normals succumbed. Since all of the pretreated animals tolerated this dose of morphine before the operation, while 2 of the non-pretreated normals receiving the same amount died, one may conclude that a certain adaptation to morphine has occurred by this time. This adaptation was decreased by removal of the adrenals, since 5 of the pretreated animals died following the operation. Still, one may conclude that this adaptation was not entirely lost after adrenalectomy, for 75 per cent of the pretreated adrenalectomized animals survived, while none survived in the adrenalectomized, non-pretreated group.

DISCUSSION

In 1913 the Belgian histologist Dustin studied the effect of certain drugs on mitotic divisions and nuclear pyknosis. More recently, he has summarized the results of his extremely interesting cytological observations (26). He states that a certain group of drugs which he terms 'poisons caryoclasiques' decreases the number of mitotic figures in the thymus and lymph glands of the mouth and increases the number of pyknoses. In a later stage, regeneration with increase in the number of mitoses occurs. He and his pupils found that definite mitotic waves may occur under the influence of these same drugs in other organs such as the liver, Langerhans' islands of the pancreas, the adrenals, the spleen and the intestinal epithelium. Cytological studies along these lines, after surgical and nervous injuries, after exposure to cold, and after the administration of sublethal doses of drugs other than the 'poisons caryoclasiques' would be necessary in order to establish the exact correlation between this apparently specific cytological reaction to certain drugs and the alarm reaction. Only if the same cytological changes could be elicited with non-specific stimuli, and on the other hand the 'poisons caryoclasiques' should produce other typical symptoms of the alarm reaction (water retention, gastro-intestinal ulcers, adrenal enlargement, drop of body temperature, etc.) and should lose their effect on the lymphatic system after adrenalectomy, would we feel justified, however, in doubting the specificity of the action of this group of substances.

In 1921 the French clinician Widal and his school gave a great deal of attention to the clinical syndrome which Widal termed 'crise hémoclasique.' This reaction was first observed by this author in cases of cold hemoglobinuria, and was later seen in numerous other clinical conditions such as acute infectious diseases, allergic conditions, etc.

The reaction has chiefly been studied from a hematological point of view. Its outstanding features are leucopenia, decrease in the refractive index of the serum, decrease in blood coagulation time, and decreased blood pressure; occasionally albuminuria and fever are also observed, and these latter are not constant symptoms. Since both Widal's reaction and the alarm reaction seem to represent responses of the organism when suddenly confronted with critical situations, a certain relationship between the two might be suspected.

The possible bearing of our observations on the theory of non-specific therapy, about which so little is known as yet, should also be mentioned.

Weichardt, in his recent monograph (27) gives an excellent review of the experimental and clinical observations made in this connection. He and Schmidt (28) developed the idea that non-specific therapy leads to the production of metabolites, which increase the efficiency of the organism and its individual organs ("Leistungssteigerung und Protoplasmaaktivierung"). It is quite likely that close correlations exist between alarming stimuli and non-specific agents.

The conception of tachyphylaxia and skeptophylaxia should also be mentioned in connection with our experiments on adaptation. In 1911 Champy and Gley (29) called attention to the fact that injection of small quantities of toxic organ extracts will protect animals against larger doses of the same extract administered shortly afterward. The name 'tachyphylaxia' has been given to this phenomenon, in order to emphasize both its possible relationship to anaphylaxia and the great rapidity with which the protective effect develops. The protection in the condition of tachyphylaxia is extremely transitory in nature and lasts for a few hours only. In the same year Ancel, Bouin and Lambert (30) described very similar results under the name of 'skeptophylaxia,' also emphasizing the transitory nature of the protection obtained by the first injection of the organ extract against subsequently administered toxic doses. It is possible that a certain relationship exists beween tachyphylaxia or skeptophylaxia and the conditions of adaptation described in this communication. Attention should be called, however, to the fact that these phylactic reactions last for a few hours only, while the adaptation which we obtained is more permanent, although we do not know as yet exactly how long it lasts.

The fact that in the course of the adaptation reaction the resistance acquired during the resistant stage gradually disappears again, so that exhaustion cccurs, suggests that the animal runs out of something which is indispensable for adaptation. The other observation—that an animal which has had to adapt itself to a certain damaging stimulus while acquiring resistance to this one loses part of its ability to resist injuries of a different nature—also suggests that something is actually used up during every process of adaptation. Since nothing is known at the time about the nature of this adaptation principle, we shall simply refer to it as 'adaptation energy.'

SUMMARY

A reaction is described under the name of 'alarm reaction' which represents a non-specific response of the organism to damage as such. Its main symptoms are: adrenal enlargement, involution of the lymphatic organs, degeneration and death of cells in various tissues, ulcer formation in the digestive tract, and edema formation. These symptoms are the same whatever the specific nature of the damaging agent may be. Drugs, surgical injuries, spinal shock, excessive muscular exercise, all elicit this same reaction.

Experiments on rats show that, if the organism is exposed daily to a stimulus causing an alarm reaction in the beginning, it will eventually become adapted to this stimulus. This adaptation, however, is lost after some time, apparently because the organism runs out of something needed for the fight against the damaging agent. At this time the animals die in a condition of

exhaustion, with symptoms similar to those seen in the acute stage of alarm. The whole reaction of adaptation consists, therefore, of 3 stages: the stage of alarm, the stage of resistance, and the stage of exhaustion. Evidence is advanced which seems to support the hypothesis that the symptoms seen in the stage of alarm are due to the liberation of a histamine-like substance.

The histological changes occurring during the reaction of adaptation are described. Adrenalectomy prevents the involution of the lymphatic organs but facilitates the production of all the other symptoms of the alarm reaction. Thyroidectomy does not cause involution of the lymphatic organs by itself, but facilitates the production of all the symptoms of the alarm reaction, including the involution of the thymico-lymphatic apparatus following exposure to damaging agents. The thymico-lymphatic apparatus of gonadectomized male and female rats does not show true hyperplasia but it approximates the upper limit of the normal, and shows no involution with advancing age. Gonadectomy does not, however, prevent the accidental involution of the thymus and the lymph glands during the alarm reaction. While adaptation is acquired to a certain stimulus, the ability to resist other stimuli decreases. The adaptation in the stage of resistance is therefore specific. The hypothesis is put forward that the factor necessary for adaptation, 'the adaptation energy,' is present in every individual in a limited quantity only.

I am greatly indebted to Professors J. B. Collip and D. L. Thomson, and to Dr. J. S. L. Browne for their stimulating criticisms which proved to be of the greatest value in conducting this work. It is a pleasure, furthermore, to take this opportunity to express my gratitude to the technicians of the department, and particularly to Mr. K. Nielsen.

REFERENCES

1. Selye, H.: Nature **138:** 32. 1936.
2. Selye, H.: Canad. M.A.J. **34:** 706. 1936.
3. Dale, H. H., and H. W. Dudley: J. Physiol. **68:** 97. 1929.
4. Cannon, W. B.: Traumatic Shock. Surgical Monographs, D. Appleton & Co., New York and London, 1923.
5. Lewis, T.: The Blood Vessels of the Human Skin and Their Responses. Shaw and Son, London, 1927.
6. Horton, B. T., G. E. Brown and G. Roth: Reported at American Medical Assoc., Kansas City, 1936.
7. Andersen, D. H.: J. Physiol. **85:** 162. 1935.
8. Anrep, G. V., and G. S. Barsom: J. Physiol. **85:** 409. 1935.
9. Ungar, G.: Compt. rend. Soc. de biol. **118:** 620. 1935.
10. Ungar, G., M. R. Zerling and A. Pocoulé: Compt. rend. Soc. de biol. **118:** 778. 1935.
11. Tinel, J., and G. Ungar: Compt. rend. Soc. de biol. **118:** 1148. 1935.
12. Opie, E. L.: Cytology of the Pancreas. In Cowdry's Special Cytology **1:** 375. 1932.
13. Herxheimer, G.: Pankreas. Hirsch's Handbuch der Inneren Sekretion **1:** 25. 1932.
14. Sun, P. T.: Anat. Rec. **34:** 341. 1926.
15. Dodds, E. C., and R. N. Noble: Nature **135:** 788. 1935.
16. Harde, E.: Compt. rend. Soc. de biol. **109:** 1326. 1932.
17. Matsueda, A.: Okayama-Igakkai-Zasshi **43:** 2973. 1931.
18. Eppinger, H., and R. Leuchtenberger: Arch. f. exper. Path. u. Pharmakol. **167:** 52. 1932.
19. Carnot, P., H. Simonnet, M. Tissier and R. Cachera: Compt. rend. Soc. de biol. **113:** 1517. 1933.
20. Brummelkamp, R.: Nederl. tijdschr. v. geneesk. **77:** 5261. 1933.
21. McIlroy, P. T.: Proc. Soc. Exper. Biol. & Med. **25:** 268. 1928.
22. Selye, H.: Brit. J. Exper. Path. **17:** 234. 1936.
23. Selye, H., C. Harlow and J. B. Collip: Endokrinologie. In Press.
24. Selye, H., and J. B. Collip: Endocrinology **20:** 667. 1936.
25. Marine, D., O. T. Manley and E. J. Bauman: J. Exper. Med. **40:** 429. 1924.
26. Dustin, A. P.: L'apport de la pathologie expérimentale à la connaissance des mécanismes cytorègulateurs. Madrid Blass S. A. Tipográphica, 1933.
27. Weichardt, W., Die Grundlagen der unspezifischen Therapie. J. Springer, Berlin, 1936.
28. Weichardt, W., and R. Schmidt: Neue Deutsche Klinik, Urban & Schwarzenberg, Berlin, 1934.
29. Champy, Ch., and E. Gley: Compt. rend. Soc. de biol. **71:** 159. 1911.
30. Ancel, Bouin and Lambert: Compt. rend. Soc. de biol. **71:** 315. 1911.

STATISTICAL SURVEY OF ENDOCRINOPATHIES AMONG YOUNG DELINQUENTS

NELSON M. TAYLOR AND ROBERT L. SCHAEFER
DETROIT, MICHIGAN

There is a marked paucity of statistical evidence of endocrine incidence in general medicine. The intent of this publication is to offer data concerning the various types of glandular imbalance among delinquent children and the increase of these delinquencies as they occur at a most important and critical period of endocrine balance, namely, adolescence. By many it has been assumed that this association represents a direct causal relationship. At this time no attempt will be made either to confirm or disprove this contention.

SOURCE OF MATERIAL AND METHOD OF STUDY

This series is composed of patients examined in the Wayne County Clinic for Child Study, a unit of the Juvenile Division of the Probate Court[1] of Wayne County, Michigan. This Clinic is unique in that it is the only known one of its type. It has been in existence in some form for 21 years. The patients are recruited directly from the actual wards of this Court, although any other recognized, protective childhood agency can by application have access to it. The geographical area comprises chiefly the metropolitan area of Detroit.

The staff consists of a director, 2 psychiatrists, a general medical examiner, an internist, a graduate nurse, 3 psychologists, 3 social workers, and 4 stenographers. The patients are usually referred by probation officers in the Court or by the other recognized agencies who wish diagnoses and recommendations for delinquent children. The case is then cleared through a central registration bureau and all other agencies known by the family are contacted by the social service worker. The home is visited, and both parents, the school teacher, principal or counsellor are interviewed. A psychiatric social history is then prepared which includes information under the following headings: referral data; problem; sources of information; informants; files consulted; hereditary factors for 3 generations; developmental history of the patient; health history; school history; court and police contacts; environment; recreation and companionship; religion; sex life; habits; personality; and family attitudes. The child is then seen by the psychologist who applies the following tests: general intelligence test of Binet; the Healy picture completion test for observation of visually presented material; the Porteus maze for concrete planning; and the Ferguson form boards for manual dexterity. During the test the examiner denotes general personality factors such as appearance, manner of working, expressed attitudes and interests and any other general information. At the discretion of the psychologist the patient is then referred for medical and psychiatric examination. When all of these tests have been completed a conference is held in which the

[1] The presiding judge is the Honorable D. J. Healy, Jr. The director of the Clinic is Miss Margaret E. Baxter.

referring agency and the staff members are present to determine a plan of corrective treatment or final disposition of the case.

Following the fundamental rule of endocrine medicine, an endocrine diagnosis is not entertained unless non-endocrine disease has been definitely ruled out. The following commonly accepted diagnostic criteria were utilized in arriving at a specific glandular diagnosis. *1) Thyroidism: a)* colloid goiter, a diffuse bilateral enlargement of the gland occurring at adolescence with a normal basal metabolic rate and no symptoms referable to the endocrine system; *b)* hypothyroidism, of the non-myxedematous or myxedematous varieties uncomplicated by other glandular disorders revealing one or more well recognized clinical signs of hypofunction with decreased metabolic rate and retardation in osseous development; *c)* hyperthyroidism, goiterous and non-goiterous showing a persistently increased basal metabolic rate together with one or more manifestations of toxicity.

2) Pituitarism: a) pituitary dwarfism was considered only when the statural measurements showed a definite but proportionate statural undergrowth distinctly below the minimal normal variations for age; *b)* gigantism, a proportionate somatic overgrowth with corresponding statural measurements definitely exceeding the maximal variations for the stated age; *c)* adiposogenital pituitarism (Fröhlich's syndrome), displaying a lack of genital development with characteristic localization of adiposity; *d)* the diagnosis of neurohypophyseal obesity was made only in females, and it was based upon a similar localization of adiposity as is seen in adiposogenital pituitarism. Lack of genital development was not positively ascertained. In all probablity more careful gynecological examination would have revealed infantile uteri resulting in their proper classification as adiposogenital pituitarism; *e)* Pituitary cachexia, or Simmonds' disease. These patients showed characteristic signs of fatigability, weight loss, decreased statural growth, and senile changes in the skin.

3) Gonadism: a) hypogonadism, revealing lack of genital development and function, delay in epiphyseal closure, relative long bone overgrowth, and alteration in mensuration (span exceeding total height and lower measurement exceeding upper measurement); *b)* hypergenitalism, evidenced by precocious signs of adolescence with genital hyperplasia, premature epiphyseal closure, and over-developed musculature.

ENDOCRINE INCIDENCE

The above study represents a survey of 2,311 delinquent children of whom 1,106 were females and 1,205 were males. Among the former, 184 or 16.7 per cent and among the latter, 208 or 17.3 per cent, accumulating to a total of 392 or 17 per cent of the delinquents, presented definite clinical evidence of endocrine disturbances. The frequency of pluriglandular dyscrasia is recognized but for the facility of classification the diagnosis which indicates the primary involvement is used.

Thyroid group. The group of cases in which the thyroid was the primary seat of disturbance comprised 40.9 per cent of all endocrinopathies and 6.7

per cent of the total delinquents. However, the female (58.6 per cent) incidence of thyroidism was more than twice that of the male (23.5 per cent). Colloid goiter represented 11.8 per cent of the total glandular disturbances, and among these the female incidence (18.4 per cent) was more than 3 times that of the male (5.3 per cent). Similarly, among the hypothyroids which constituted 27.3 per cent of the total endocrinopathies, the rate of female incidence (36.4 per cent) was exactly twice that of the male (18.2

TABLE 1. ENDOCRINOPATHIC INCIDENCE IN YOUNG DELINQUENTS, WARDS OF PROBATE COURT, WAYNE COUNTY, MICHIGAN.

	Female 1,106 cases			Male 1,205 cases			Total 2,311 cases		
	Number of cases	Per cent of all endocrinopathies	Endocrine cases as per cent of all delinquents	Number of cases	Per cent of all endocrinopathies	Endocrine cases as per cent of all delinquents	Number of cases	Per cent of all endocrinopathies	Endocrine cases as per cent of all delinquents
Thyroidisms	108	58.6	9.8	49	23.5	4.1	157	40.9	6.7
Colloid goiter	34	18.4	3.1	11	5.3	1.0	45	11.8	1.9
Hypothyroid	67	36.4	6.0	38	18.2	3.1	105	27.3	4.5
Nodular hyperthryroidism	2	1.1	0.2				2	0.5	0.1
Exopthalmic goiter	5	2.7	0.5				5	1.3	0.2
Pituitarisms	64	34.8	5.8	117	56.1	19.7	181	45.4	7.9
Pituitary dwarfism	12	6.5	1.1	67	32.2	5.6	79	19.3	3.5
Pituitary cachexia				4	1.9	0.3	4	1.0	0.2
Adiposogenital pituit.	19	10.4	1.7	28	13.4	2.3	47	12.0	2.0
Neurohypophyseal obesity	23	12.5	2.1				23	5.9	1.0
Gigantism	10	5.4	0.9	18	8.6	1.5	28	7.2	1.2
Gonadisms	12	6.6	1.1	42	20.4	3.5	54	13.7	2.4
Hypogonadism	8	4.4	0.7	27	13.0	2.3	35	8.9	1.5
Pseudo-hermaphroditism				2	0.9	0.2	2	0.5	0.1
Cryptorchism				4	1.9	0.3	4	1.0	0.2
Hypergenitalism	4	2.2	0.4	9	4.6	0.7	13	3.3	0.6
Total endocrine	184	100.0	16.7	208	100.0	17.3	392	100.0	17.0
Total probable endocrine	58			105			163		

per cent). The few cases of adolescent hyperthyroidism were all in girls.

Pituitary group. The group of cases in which the pituitary was considered the gland of primary etiological significance comprised 7.9 per cent of the delinquents and 45.4 per cent of all endocrinopathies. However, in contrast to the thyroid situation, the largest number of these was among males (56.1 per cent of male endocrinopathies), almost twice as many as among girls. Pituitary dwarfism accounted for the greatest number of pituitary involvements among these children. In this class fell 32.2 per cent of the male, but only 6.5 per cent of the female endocrinopathies. The 4 cases (1.9 per cent) of pituitary cachexia (Simmonds' syndrome) were all in males. Adiposogenital pituitarism accounted for 12 per cent of the total endocrinopathies;

13.4 per cent of the male and 10.4 per cent of the female deviators composed this group. Neurohypophyseal obesity included only girls, representing 12.5 per cent of their dyscrasias and 5.9 per cent of the total. Gigantism included 5.4 per cent of the female and 8.6 per cent of the male endocrinopathies to account for 7.2 per cent of the total.

Gonad group. The group of cases in which the gonads were considered primary offenders constituted 13.7 per cent of all the endocrinopathies and 2.4 per cent of the total delinquents. Among these the males presented an incidence (20.4 per cent) of approximately 3½ times greater frequency than did the females (6.6 per cent). Hypogonadism accounted for 8.9 per cent of the total dyscrasias. The male incidence (13 per cent) approximately tripled that of the female (4.4 per cent). Adding pseudohermaphroditism (0.9 per cent) and cryptorchism (1.9 per cent) to this male group the disparity is enhanced to almost 4 times. The group showing hypergenitalism (macrogenitosomia) was 3.3 per cent of the total number. The male incidence (4.6 per cent) was about double that of the female (2.2 per cent).

Probable endocrinopathies. In the classification of probable endocrinopathies are included: *1)* cases in which preliminary studies indicated clinical evidences of endocrine deviation but in which the patients did not return to permit absolute establishment of the diagnosis; *2)* cases which are at present under observation for anticipated endocrinopathies; *3)* cases in which the gland of primary etiological significance was not one of the 3 noted above, and which comprised such a small group as to be statistically unimportant in this series.

AGE INCIDENCE OF DELINQUENCY

Another interesting observation is the age incidence of delinquents and the relative incidence of the endocrinopathies among the delinquents. It has been generally assumed that the greatest number of delinquencies occur at the age of puberty, but the marked increase in the number of behavior problems at this period is startling.

The graphs in figure 1 represent in percentages the number of delinquents in general (solid line) and the number of endocrinopathic delinquents (broken line) which were referred to us at the various ages. For convenience, the cases under 10 years of age are grouped, as are those over 17 years.

Among the total female delinquents there was a gradual rise from 2.3 at 10 years to 7.7 at 13 years and then an abrupt increase to 13.3 at 14 with continued increase to 16.1 per cent at 15 years. At 16 years the incidence was 15.1 per cent, following which there was an abrupt decline to 8.4 per cent at 17 years.

Among the female glandular delinquents the rate at 10 years was 1.8 with a very gradual increase to 4.8 at 12 years and then a relatively abrupt rise to 13.7 at 13 years which continued to 19 at 14 years, and 20.2 per cent at 15 years. At 16 years the incidence was 16.1 and at 17 it was 11.3 per cent.

The incidence of delinquency in the total male group studied started at 6.9 at 10 years, was 6.7 at 11 years, followed by a gradual elevation to 10.6

at 12, 14.6 at 13, and 15.3 per cent at 14 years. There was then a constant decrease to 12.7 at 15, and 9.3 at 16; after which there was an abrupt fall to 1.4 per cent at 17 years.

The endocrinopathic males showed an incidence of 2.6 at 10 years followed by a constant, though steep inclination to 8.4 at 11, 14.1 at 12, and 19.9 per cent at 13 years. There was then a slight fall to 17.8 per cent at 14 years. Another slight increase to 20.9 at 15 and a steep declination to 6.8 at 16 and 3.1 per cent at 17 years was noted.

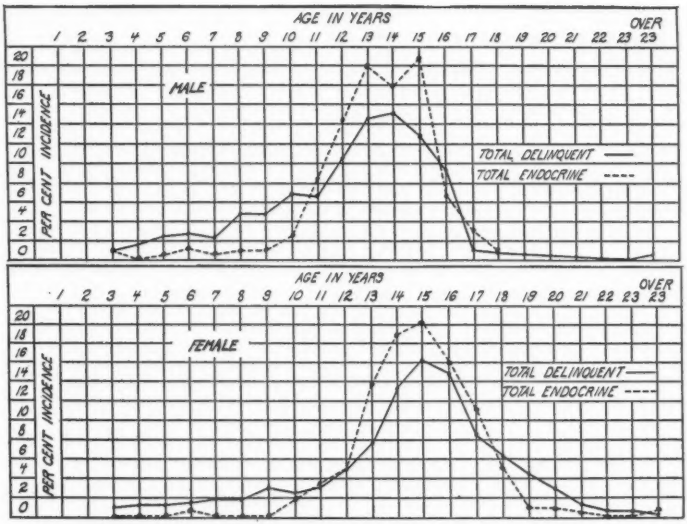
AGE IN YEARS
OVER
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 23
20 18 16 14 12 10 8 6 4 2 0
PER CENT INCIDENCE
MALE
TOTAL DELINQUENT
TOTAL ENDOCRINE
AGE IN YEARS
OVER
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 23
20 18 16 14 12 10 8 6 4 2 0
PER CENT INCIDENCE
FEMALE
TOTAL DELINQUENT
TOTAL ENDOCRINE

Fig. 1. Behavioristic problems as related to adolescence. Age incidence of delinquency in males and females.

COMMENT

It is interesting to note the relatively even sex distribution in the group of 2,311 behavior problem children. There were only 4 per cent more males than females. Similarly, there was a fairly equal total distribution of endocrinopathies between the sexes. The incidence of glandular disturbance among the boys was only 0.6 per cent more frequent than among the girls.

If, however, the individual endocrinopathies are considered, it is seen that the females accounted for the greatest number of thyroid disturbances, while the males constituted the greater percentage with pituitary and gonad involvement. Hypothyroidism accounted for the greatest number of thyroid disturbances, and, incidentally, composed the largest number of cases included in a single endocrinopathy. There were, however, relatively twice as many females in this classification as males. The largest number of males in a single classification was among the pituitary dwarfs, where they outnumbered the females almost 5 to 1.

An explanation for the increased frequency of thyroidism in the female

can not be arrived at as this condition should be as easily reached in one sex as the other. The same is true of pituitary dwarfism in which the males predominate. A seemingly plausible explanation for the increased frequency of gonadism in the male is the fact that the genitalia are external and easily inspected as to size and development; whereas it is much more difficult to determine this in the female.

The graphic demonstration of age incidence of delinquency displays the very marked increase in number of behavior problems encountered during the period of puberty. Among the delinquents who are also endocrinopaths the rise is much greater. This single study opens up vast avenues of conjecture as to causal relationships between the abnormal endocrine function and abnormal social behavior, but, as previously stated, we shall attempt no such evaluation.

SUMMARY AND CONCLUSIONS

Statistical evidence on a group of adolescent delinquents is presented, not to establish causal relationships between endocrine dysbalance and behavior problems, but to demonstrate the association. The method of study of the cases in this group is outlined. The group included 1,106 females and 1,205 males, a total of 2,311 delinquent adolescents. The percentage of endocrinopathies was almost evenly distributed between the sexes with a total of 17 per cent, which is 7 per cent greater frequency than Engelbach's[2] estimated glandular dysbalance in the general population.

The greatest number of cases occurred in the pituitary group. Hypothyroidism accounted for the greatest number of cases in a single endocrinopathy and also represented the greatest percentage of female endocrinopathies. Pituitary dwarfism was the classification which included the greatest number of males. Gonadism comprised about 14 per cent of the total glandular disturbances and included almost 3½ times as many males as females. If the group of probable endocrinopathies is added to the positively diagnosed cases, it raises the percentage to 24 per cent, or 140 per cent increase over Engelbach's estimation of glandular dysbalance in the total population. The age incidence graphs demonstrate: *a)* a marked increase in delinquency during the "difficult period" of puberty; *b)* the more acute numerical elevation and higher incidence among the endocrinopaths at the time of puberty.

An analysis of the figures presented reveals a graphic demonstration that behavioristic problems are much more prominent during the years of adolescence and that these behavioristic problems are definitely increased if the adolescent child has an associated endocrine imbalance.

1304 Kales Building
Detroit, Michigan

[2] Engelbach, William: Endocrine Medicine. Charles C Thomas, Springfield, 1932.

OBSERVATIONS ON 'PREDIABETES'

HUGO R. RONY
From the Medical Department, Northwestern University Medical School
CHICAGO, ILLINOIS

Abnormal blood sugar curves indicating 'low sugar tolerance' may be found in normal persons at times of emotional upset (1) or while they are living on low carbohydrate diets (2). Advanced age (3), high blood pressure (4), liver or kidney insufficiency (5, 6) tend to decrease the glucose tolerance. In addition, there are some persons, young or in middle age, in apparently good health, with blood pressure normal or slightly higher, without demonstrable liver or kidney disturbance, living on unrestricted normal diet who upon glucose tolerance tests consistently respond with markedly abnormal blood sugar time curves approaching those found in the diabetic. Such persons are almost invariably obese. Paullin and Souls (7) first called attention to this condition; John (8) called it 'prediabetes.' Rony (9) found it in 16 of 70 unselected cases of adult obesity.

The frequent occurrence of this condition in obesity deserves attention for several reasons. In the first place there is the question whether or not obese 'prediabetics' are predisposed to diabetes. John, and Paullin and Souls believed that such is the case, and that the disease can be prevented by reducing the subject's weight by appropriate diet. No direct evidence was furnished, however, as to the reality of the danger of diabetes or its actual abolition by reduction of weight. In the second place 'prediabetes' in obesity is of interest because of its possible bearing on certain problems concerning the intermediate carbohydrate metabolism in obesity. This work was undertaken with a view to obtaining information on these practical and theoretical questions.

In the selection of material the procedure was as follows. Cases of marked obesity in apparent good health and with essentially negative physical and laboratory findings were subjected to a glucose tolerance test. This was made in the morning, on empty stomach, 120 gm. of glucose being given with 2 glasses of water. Capillary blood was taken before the ingestion of glucose, and ½, 1, 2 and 3 hours following it. Determination of sugar in the blood was made by the Folin-Wu method. Urine specimens were obtained at the same periods and tested with Benedict's qualitative reagent. If the blood sugar time curve indicated 'prediabetes,' the test was repeated after 1 to 2 weeks, the patient being instructed to continue to live on unrestricted diet in the meantime; the patient was classed definitely in the 'prediabetic' group if the results of the 2 tests were practically identical.

Two groups of cases were then selected. *Group A),* 'prediabetes,' included cases with at least one postingestive blood specimen showing more than 300 mg. per cent blood sugar; cases with at least 3 blood specimens above 200 mg.

per cent; and cases with at least 2 specimens above 200 mg. per cent with the 3-hour specimen above 180 mg. per cent. *Group B),* 'normal sugar tolerance,' included patients whose maximum postingestive blood sugar was below 180 mg. per cent, the 3-hour specimen falling below 120 mg. per cent. The patients were advised to restrict their caloric intake in order to lose weight; no other treatment was given. Subsequent glucose tolerance tests with the same amount of glucose were made once or twice yearly.

Group A. Twenty obese 'prediabetics' were observed for from 1 to 9 years; the average observation time was 3.0 years. The age of the patients varied between 16 and 50 years, the average age being 38; 18 were women, 2 were men. The body weights ranged between 180 and 310 lbs., with an

TABLE 1. SHOWING RELATIONSHIP BETWEEN CHANGE IN GLUCOSE TOLERANCE AND CHANGE IN WEIGHT, IN 20 OBESE "PREDIABETICS." AVERAGE OBSERVATION TIME 3 YEARS. THE FIGURES REPRESENT NUMBER OF CASES.

		Glucose Tolerance		
		increase	no change	decrease
Weight	decrease	12	2	—
	no change	2	3	—
	increase	1	—	—

TABLE 2. SHOWING INCREASE IN GLUCOSE TOLERANCE IN 15 OBESE "PREDIABETICS." AVERAGE OBSERVATION PERIOD 3 YEARS.

	Average blood sugar, mg. per cent				
	fasting	½ hr.	1 hr.	2 hr.	3 hr.
Initial test	130	219	280	246	198
Last test	92	166	202	172	116

average of 238 lb. Their excess weight varied from 52 to 175 lb., the average excess weight being 101 lb. The highest systolic blood pressure observed in this group was 180 mm., the lowest was 105, and the average was 148. The basal metabolic rates varied between —21 per cent and +19 per cent, the average being +4 per cent. The menstrual history was normal in 8 cases; 6 patients had irregularly delayed menstrual periods and 3 had passed the menopause. In 3 cases there was a history of maternal diabetes.

By the end of the observation period 14 patients had lost more than 5 per cent of their original weight; the average loss of weight in these patients was 25.5 lb. The weight of 5 patients remained within ±5 per cent. One patient's weight increased 18 per cent. The average systolic blood pressure at the end of the observation was 140 mm.

The data are summarized in tables 1 and 2. Figure 1 illustrates some of the results. It is seen from the tables that: *a)* the glucose tolerance increased during our observation in the majority of the cases; *b)* permanent decrease did not occur in any case; *c)* there is a significant, albeit not absolute, relationship between increase in glucose tolerance and loss of weight. As a rule, the increase

in glucose tolerance was noticeable soon after some loss of weight, and the improved tolerance was maintained throughout or improved further as time went on. A temporary decrease in glucose tolerance occurred in one case. No patient developed diabetes. Three cases are described in some detail as they present unusual features.

Miss B.O., 16 years old, 64 per cent overweight. Two years after admission patient was married and had 3 children in the following 5 years.

On admission	weight	180 lb.	; glucose	tolerance	118; 245, 267, 286, 260
2 weeks later	"	181 "	"	"	95; 250, 266, 308, 266
6 months later	"	162 "	"	"	111; 220, 241, 210, 189
4 years later	"	192 "	"	"	105; 168, 161, 162, 130
9 years later	"	212 "	"	"	61; 164, 184, 147, 100

This is the only patient whose weight increased; at the same time the glucose tolerance increased. (This is case 3 in fig. 1.)

Mrs. E.B., 34 years old, 60 per cent overweight.

On admission	weight	180 lb.	; glucose	tolerance	133; 186, 208, 266, 222
1 year later	"	195 "	"	"	122; 250, 267, 260, 190
4 years later	"	186 "	"	"	143; 250, 308, 273, 251
5 years later	"	178 "	"	"	109; 143, 227, 236, 174

This is the only case in which a (temporary) decrease of the sugar tolerance occurred.

Mr. L.Y., 50 years old, 65 per cent overweight.

On admission	weight	240 lb.	; glucose	tolerance	187; 231, 345, 200, 44
2 weeks later	"	234 "	"	"	105; 245, 298, 222, 42
1 year later	"	201 "	"	"	67; 169, 212, 200, 48

There was a marked hypoglycemic reaction at the end of each test. The unusual feature in this case is the association of a 'prediabetic' state with hypoglycemia, following ingestion of glucose. (This is case 4 in fig. 1.)

All cases showed glycosuria during the initial test in at least one of the urine specimens obtained after ingestion of the glucose. Only 9 had glycosuria

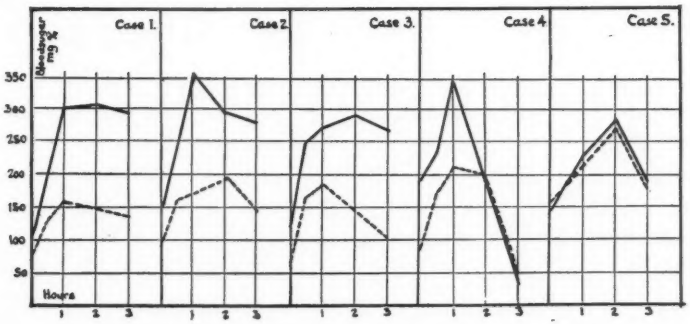

Fig. 1. Changes in glucose tolerance in obese 'prediabetics'. Solid line, blood sugar curve at time of admission. Broken line, curve at end of observation period, as follows: case 1, 3 years; case 2, 1 year; case 3, 9 years; case 4, 1 year; case 5, 2.5 years.

during the test at the end of the observation period. Of the 3 patients whose family history revealed diabetes of the mother, 1 lost 16 lb. and showed marked improvement of glucose tolerance after 3 years (case 1 in figure 1); in the 2 others weight and glucose tolerance remained unchanged after 2.5 and 4 years, respectively (see case 5 in figure 1).

Group B. Eight obese women with normal glucose tolerance were observed

in this group for 1 to 6 years; the average observation time was 4.0 years. The age of the patients varied between 29 and 45 years; average age 37.5 years. Body weight ranged from 231 to 315 lb.; average weight 270 lb. The excess body weight was between 93 and 167 lb.; average excess weight 135 lbs. Systolic blood pressures varied from 100 to 230 mm.; with an average of 159. Basal metabolic rates varied from —7 per cent to +27 per cent; the average was +6 per cent. Menstruation was regular in 2 cases, irregular and scant in 4 cases; there was amenorrhea in 1 case and hypermenorrhea in 1 case. The family history was reported free from diabetes in every case in this group.

By the end of the observation period 4 patients lost more than 5 per cent

TABLE 3. SHOWING CONSTANCY OF GLUCOSE TOLERANCE IN 8 OBESE PERSONS WHOSE GLUCOSE TOLERANCE ORIGINALLY WAS NORMAL. AVERAGE OBSERVATION PERIOD 4 YEARS.

	Average blood sugar, mg. per cent				
	fasting	½ hr.	1 hr.	2 hr.	3 hr.
Initial test	90	177	167	127	94
Last test	84	153	166	142	73

TABLE 4. EFFECT OF DIET ON THE GLUCOSE TOLERANCE OF OBESE "PREDIABETICS." VALUES ARE FOR BLOOD SUGAR, IN MG. PER CENT.

Case	Unrestricted diet					Undernutrition					High carbohydrate diet				
	fasting	½ hr.	1 hr.	2 hr.	3 hr.	fasting	½ hr.	1 hr.	2 hr.	3 hr.	fasting	½ hr.	1 hr.	2 hr.	3 hr.
1	84	200	255	224	136	84	137	158	135	120					
2	150	190	242	262	182	102	123	188	153	122	166	171	206	212	177
3	122	188	224	280	186	85	139	192	111	133	81	148	286	203	123
4	111	222	202	200	103	91	154	130	118	103					
5	80	184	225	230	213	84	203	205	191	111					
6	153	170	226	282	280	138	171	224	267	169					
7	109	143	227	236	201	108	155	210	207	160	105	210	278	262	207

of their original weight; the average loss of weight was 38 lb. The weight of the 4 other patients remained within ±5 per cent. As seen in the summarized results (table 3) the average glucose tolerance showed no material change at the end of our observation. This is also true for each individual case.

It is known (10) that normal persons and animals fasted or fed a low carbohydrate, high fat diet show immediate marked decrease in glucose tolerance; low fat, high carbohydrate diet, on the other hand, causes prompt increase in glucose tolerance. Himsworth (11) has conclusively shown that these acute effects of the diet are due solely to its carbohydrate content the caloric value or fat content having no direct role in producing these effects. We have seen that the obese 'prediabetic' responds in an opposite way to chronic underfeeding. In order to compare on an equal basis, we extended our study of the glucose tolerance of the obese 'prediabetic' to an examination of the effect of acute underfeeding. Six such patients, following a period of unrestricted

eating, were put on a diet consisting of 3 glasses of milk, 2 glasses of orange juice and 2 glasses of tomato juice daily—approximately 26 gm. protein, 24 gm. fat, 75 gm. carbohydrate, equal to about 650 calories—for a week. One additional case fasted completely for 72 hours. All patients lost 4 to 6 lb. by the end of the undernutrition perod. Just before, and immediately after this period glucose tolerance tests were made. In 3 cases the undernutrition period was immediately followed by a high carbohydrate period, the diet containing approximately 80 gm. protein, 50 gm. fat and 300 gm. carbohydrate, daily for a week; a third glucose tolerance test was made the following morning. The results are shown in table 4.

It is seen that acute undernutrition carried out in the manner stated caused more or less marked increase in the glucose tolerance in every case. After the high carbohydrate period, marked decrease occurred in all 3 cases. It follows that, insofar as glucose tolerance is concerned, the obese 'prediabetic' responds to acute undernutrition and high carbohydrate diet in a characteristic way which is the opposite of that shown by normal persons.

Finally, the response to acute undernutrition was examined in 2 obese patients having normal glucose tolerance. 1) Mrs. L. P., 27 years old, 92 lb. overweight. A glucose tolerance test after a period of unrestricted diet resulted in the following blood sugar values: 77; 129, 180, 140, 122. After 3 days complete fasting, with a loss of 3.5 lb. the test was repeated with the following result: 64; 120, 164, 143, 107. 2) Mrs. A. A., 42 years old, 105 lb. overweight. After an unrestricted diet the blood sugar values were: 82; 133, 178, 135, 119. After 3 days fasting with a loss of 6 lb. the results were: 54; 125, 152, 169, 115. It is seen that fasting caused no material difference in the sugar tolerance of these cases.

COMMENT

The high incidence of obesity in diabetes indicates that some relationship exists between these two conditions. Although the nature of this relationship is far from being clearly understood, it is commonly believed that obesity creates in some way a predisposition for diabetes (2). In consequence of this contention the presence of low sugar tolerance in obese persons has come to be regarded as a danger sign of diabetes (prediabetes). This prognostic evaluation is the more conspicuous as low sugar tolerance in other clinical conditions is, as a rule, not thought to be conducive to diabetes.

In this study we have undertaken to keep obese patients with very low sugar tolerance under observation in order to find out when and under what conditions manifest signs and symptoms of diabetes would appear. We have followed 20 such persons for 1 to 9 years and found that none of them developed diabetes. As the patients received instructions to follow a low caloric diet, one might think that the undernutrition was instrumental in preventing the development of diabetes. However, at least 6 of our patients failed to restrict their food intake, judging from the fact that their weight remained constant or increased. It appears therefore that low sugar tolerance observed in obese persons is not, in itself, indicative of a danger of diabetes. In this

respect low sugar tolerance in obesity does not seem to differ from the low sugar tolerance observed in other conditions.

We did find, however, a notable difference between obese persons with low sugar tolerance and normal persons in regard to their response to undernutrition: the former invariably show a marked improvement of their sugar tolerance while the latter are known to respond with a marked decrease of sugar tolerance. We suggest that this peculiar difference may be due to the role of the fat tissue in blood sugar regulation—a factor which hitherto has not received due consideration. Cori and Goltz have shown (12) that by far the largest part of intravenously injected glucose disappears from the blood stream within a few minutes. It is not the liver that removes the sugar at this time, because the glycogen content of the liver is not increased until after 3 hours following glucose feeding (13). Accordingly, during the first 3 hours following a glucose test meal glucose accumulation in tissues other than the liver constitutes the major mechanism of blood sugar regulation. There is uncontested evidence that among other tissues the fat tissue may prominently participate in the mechanism of blood sugar removal after glucose ingestion. It has been shown that the fat depots regularly contain 0.2 to 0.4 per cent glycogen, and occasionally as much as 2 to 7 per cent glycogen within 2 hours after abundant carbohydrate feeding, provided that the feeding was preceded by fasting, although no glycogen is found in the fat tissue under normal feeding conditions or after excessive carbohydrate meals consumed by normally fed persons or animals (14). In other words previous undernutrition tends to make the fat tissues capable of absorbing large amounts of sugar from hyperglycemic blood. In applying this knowledge to our observations we recall that our obese patients had 52 to 175 lb. excess fat tissue. This fat tissue was not actively participating in the removal of blood sugar when the glucose test meal was taken without previous undernutrition. However, when the test was repeated after a period of undernutrition, the fat tissue became capable of accumulating glucose in the form of glycogen. Assuming that the total amount of administered glucose, 120 gm., is temporarily held this way by the excess fat tissue alone, the glycogen content of these fat tissues would amount to less than 0.4 per cent, i.e., less than what is usually found in the fat depots of normal men and animals under similar experimental conditions. Even the removal of only a part of the administered glucose by the fat tissues of the obese after undernutrition must cause a flattening of the blood sugar time curve as compared with that obtained in the same person under normal nutritional conditions when the fat tissue does not absorb glucose.

We believe that this interpretation sufficiently explains the phenomenon in question. The basic effect of undernutrition on carbohydrate metabolism—be it to decrease insulin sensitiveness (11) or something else—may be the same in normal as in obese persons. In individuals with small amounts of fat tissue this effect appears in the form of decreased glucose tolerance. In persons with large amounts of excess fat tissue the same effect is compensated by accumulation of carbohydrate in the fat tissue, with the result that the blood sugar time curve becomes flatter, giving the picture of improved glucose tolerance.

SUMMARY

Twenty cases of 'prediabetes' in obese persons have been observed for 1 to 9 years. None of them developed diabetes. The glucose tolerance increased in the majority of cases, and remained on the same level in others; persistent decrease was not observed. The changes in glucose tolerance were significantly but not invariably related to the changes in body weight. There was no direct evidence that low sugar tolerance in obesity was a precursor to diabetes; the term 'prediabetes' is prejudicial and not warranted.

Obese persons with low glucose tolerance respond to low carbohydrate diet or fasting with immediate increase of glucose tolerance; high carbohydrate diet promptly depresses the glucose tolerance. This is the opposite of what is known to occur in normal persons.

It is suggested that these differences between obese and normal persons are due to the capacity of the fat tissue to absorb large amounts of glucose from the blood when glucose is ingested after a period of undernutrition.

REFERENCES

1. Folin, O., and H. Berglund: J. Biol. Chem. **51:** 213. 1922.
2. Joslin E. P.: The Treatment of Diabetes Mellitus p. 111. Lea and Febiger, Philadelphia, 1935.
3. Marshall, F. W.: Quart. J. Med. **24:** 257. 1931.
4. Musser, J. H., and D. O. Wright: J.A.M.A. **101:** 420. 1933.
5. Althausen, T. L., E. Thoenes.: Arch. Int. Med. **50:** 257. 1932.
   Soskin, S., M. D. Allweis, and D. J. Cohn. Am. J. Physiol. **109:** 155. 1934.
6. Gray, H.: Arch. Int. Med. **31:** 241. 1923.
7. Paullin, J. E., and H. C. Souls. South. M. J. **15:** 249. 1922.
8. John, H. J.: Am. J. M. Sc. **173:** 184. 1927.
9. Rony, H. R.: Endocrinology **16:** 601. 1932.
10. Sweeney, J. S.: Arch. Int. Med. **40:** 818. 1927.
    Himsworth, H. P.: J. Physiol. **81:** 29. 1934.
11. Himsworth, H. P.: Dietary factor determining sensitivity to insulin of healthy men, Clin. Sc. **2:** 67. 1935.
12. Cory, C. P., and H. L. Goltz: Proc. Soc. Exper. Biol. & Med. **23:** 124. 1926.
13. Gigon, A.: Zur Kenntnis des Kohlenhydratstoffwechsels und der Insulinwirkung, Ztschr. f. klin. Med. **101:** 17. 1925.
14. Arndt, H. J.: Beitr. z. path. Anat. u. z. allg. Path. **79:** 69. 1927.
    Schur, H., and A. Loew.: Wien. klin. Wøchnschr. **41:** 261. 1927.
    Hoffman, A., and E. Wertheimer.: Arch. f. d. ges. Physiol. **217:** 728. 1927.

THE EFFECT OF CORTIN ON THE SODIUM, POTASSIUM, CHLORIDE, INORGANIC PHOSPHOROUS AND TOTAL NITROGEN BALANCE IN NORMAL SUBJECTS AND IN PATIENTS WITH ADDISON'S DISEASE[1]

GEORGE W. THORN, HELEN R. GARBUTT, FRED A. HITCHCOCK AND FRANK A. HARTMAN

From the Department of Physiology, The Ohio State University
COLUMBUS, OHIO

This investigation was undertaken in an effort to determine the usefulness of electrolyte and nitrogen balance studies in the diagnosis of early Addison's disease. Three classes of subjects were used in the study, normal individuals, patients with severe Addison's disease and patients who presented some of the signs and symptoms usually associated with Addison's disease. In all, the influence of cortin on these balances has been determined.

Patients with Addison's disease do not lend themselves well to metabolic investigation. This fact coupled with the limited amount of potent adrenal cortical extract which has been available in the past is largely responsible for the few balance studies which have been made in this disease.

METHODS

Normal subjects. The diet and liquid intake were constant. The food was purchased in 5 day lots according to the method of Atchley et al (1). Duplicate samples representing one half of one day's diet of each period were weighed, dried and analyzed. The diet was so selected that it theoretically approached neutrality. Distilled water was used for drinking, cooking and for washing dishes. The subjects were permitted to carry on their regular activities under supervision. The control and cortin periods were conducted during similar days of the week so that variations in daily activity would be matched. The study was made in Columbus, Ohio, during December 1935. In the control and cortin periods additional sodium chloride was given in the form of an intravenous infusion of approximately 500 cc. of 2 per cent NaCl. The amount given to each individual was carefully weighed and the time consumed for injecting the solution was the same in both periods. One infusion was given during the control period and again on the same day of the week during the cortin period (4 days after cortin injections had been started). During the treatment period subcutaneous cortin injections (2 cc.) were given 2 or 3 times daily, amounting to 12 or 18 cat units of cortin per day. On the fifth day of the control and cortin periods with the subject in a fasting state and immediately preceding the intravenous infusion, venous blood specimens were drawn under oil with as little stasis as possible. The balance experiments were conducted during the intermenstrual period of the 3 female subjects to obviate as far as possible the marked fluctuation in electrolyte excretion which occurs in some women at or near

[1] Aided by a grant from The Rockefeller Foundation.

catamenia (unpublished data). Plasma sodium, chloride and serum potassium determinations were made. Twenty-four hour urine specimens were collected, preserved with tolueen and kept at 4°C. The feces were collected daily but analyzed in 3 or 5 day periods. The determination of sodium in blood plasma, urine, feces and diet was made according to the method of Butler and Tuthill (2). Plasma chloride was determined according to the adsorption indicator method as described by Saifer and Kornblum (3). Determination of chloride in urine, diet and feces was made by the open Carius method using a modification of the Volhard-Harvey titration (4). The method of Hubbard was used for determining potassium in blood serum, diet, feces and urine (5, 6). The diet, urine and fecal nitrogen determinations were made according to the macro-method of Kjeldahl.

Two subjects, who did not receive cortin, were studied at the same time as the group of 8 treated normal subjects. These 2 subjects served as control for changes which might occur during the experiment as a result of altered atmospheric conditions.

Patients with Addison's disease. Shortly after admission of the patient to the metabolic ward, treatment was discontinued for 6 or more days depending upon the change in the patient's condition. Cortin treatment (6 C.U. 4 times daily, injected subcutaneously) was then instituted and continued until an appreciable change in electrolyte balance had occurred. During each experiment the blood pressure was recorded twice daily and the body weight was determined each morning before breakfast. The patients were kept at rest during the entire experiment. Intravenous infusions of sodium chloride were administered to patients E.K. and O.B. The diet, liquid intake, collection and analyses of specimens were similar to those described for normal subjects with the single exception that distilled water was not used in the balance studies of patients with Addison's disease.

CASES

Case 1. Patient C.B. a white male, 35 years old, had been in good health until June 1935 at which time he first noted asthenia, weakness, anorexia and weight loss. These symptoms progressed until it became difficult for the patient to carry on his duties as mail carrier. During the Christmas holiday season (1935) the patient was forced to give up his work and was confined to bed. During this period nausea and frequent vomiting were experienced. The family noted marked restlessness and irritability. The patient became progressively weaker and was finally admitted to the University Hospital Feb. 15, 1936 in a semicomatose state, delirious and obviously suffering from circulatory collapse.

The blood pressure at this time was 50/15, the pulse rate 100 per minute and the heart sounds distant. Cramps involved the muscles of the abdomen and extremities. The only area of increased pigmentation occurred on the forehead where the patient's cap had rubbed. X-ray examination of the sella turcica, region of the adrenals, and gastrointestinal tract revealed no abnormality. A general fibrosis of the lungs was noted.

On admission to hospital the red cells numbered 3,000,000 and the white cells 11,500, there being 56 per cent polymorphonuclears, 20 per cent lymphocytes, 16 per cent monocytes and 5 per cent eosinophils. The hemoglobin was 10 gm. per 100 cc. Urinalysis was negative. The values for 100 cc. of blood were 13 mgm. of urea, 22 mgm. of non-protein nitrogen and 85 mgm. of dextrose.

Case 2. Patient R.R., a housewife, 41 years old, first noticed in 1930 weakness associated with increasing pigmentation. The pigmentation was most marked on the

lips, gums and mucous membranes of the mouth. In 1934 the weakness became very marked and was associated with anorexia and weight loss. Cortin treatment was begun in 1935 with marked benefit and has been continued intermittently since.

The patient was admitted to the University Hospital on 4-16-36. At this time the blood pressure was found to be 84/56, the pulse rate 80 per minute and the oral temperature 98°F. The patient was almost bronze in color, especially in areas over the arms, legs and face. The nails were pigmented as well as the folds in the palms of the hands. The mucous membranes of the mouth, lips and gums were deeply pigmented. X-ray examination of the sella turcica and region of the adrenals gave normal findings. A slight infiltration was noted in the right apex which suggested the possibility of early tuberculosis.

Urinalysis gave normal findings. The red blood cells numbered 4,870,000 and the white blood cells 3,700, there being 30 per cent polymorphonuclears, 50 per cent lymphocytes, 10 per cent monocytes, 4 per cent basophils and 6 per cent eosinophils. The values for 100 cc. of blood, were 14 mg. of urea and 86 mg. of dextrose.

Case 3. Patient I.B. a housewife, 42 years old had been in good health until 1930 at which time she complained of asthenia, anorexia and weight loss. The symptoms increased slowly in intensity and were accompanied by attacks of dizziness which were most marked on arising suddenly. In 1934 the patient first noted an increase in pigmentation on the shoulders and hips. During 1935 treatment with cortin and NaCl resulted in improvement. The patient had received no treatment since March 1936. From March 1936 until May 1936 the patient had been confined to bed.

On admission to the University Hospital, 5-22-36, the patient's weight was 43.1 kg., blood pressure 104/70, pulse rate 96 per minute. There was evidence of marked weight loss and dehydration and the skin was generally pigmented, being a dark bronze color. The pigmentation was especially marked over the extensor surfaces of the body, palms of the hands, nails and genitalia. X-ray examination of the sella turcica and region of the adrenals revealed no abnormality. Both lung fields were found to be involved in a diffuse fibrosis without evidence of specific pathology.

Urinalysis gave normal findings. The blood urea was 13.5 mg. per 100 cc. and the blood dextrose 93 mg. per 100 cc. The hemoglobin value was 12.5 gm. per 100 cc. The red blood cells numbered 3,420,000 and the white blood cells 8,700, there being 63 per cent polymorphonuclears, 26 per cent lymphocytes, 9 per cent monocytes and 2 per cent eosinophils.

Case 4. Patient E.K., a white female, 42 years old, first noted an increase in pigmentation in 1933. This was associated with muscular weakness, gastrointestinal disturbance and weight loss. Two years ago an x-ray examination indicated pulmonary tuberculosis of the right upper lobe of the lung. During the past 2 years the patient improved considerably with rest and small doses of a glycerol extract of the adrenal cortex by mouth. Treatment was intermittent, and she was able to carry on limited activity. On 1-11-36 this patient was admitted to the University Hospital for special balance study to determine, if possible, whether she actually had early Addison's disease.

On admission the patient weighed 41.3 kg., the pulse rate was 80 per minute and the blood pressure 110/70. The skin was deeply pigmented especially about the buttocks, the upper and inner aspects of the thighs and the perineal region. Examination of the lungs revealed persistent, fine rales over the right apex with impaired resonance over the right apex posteriorly. X-ray examination of the sella turcica

and region of the adrenals gave normal findings. An arrested lesion of the upper lobe of the right lung was noted.

Urinalysis gave normal findings. The red blood cells numbered 4,520,000 and the white blood cells 6,900 there being 58 per cent polymorphonuclears, 27 per cent lymphocytes, 12 per cent monocytes and 3 per cent eosinophils. The non-protein nitrogen of the blood was 21 mg. per 100 cc. and the blood dextrose 100 mg. per 100 cc. The carbon dioxide combining power of the blood was 42 volumes per cent. The concentration of blood plasma sodium was 139.0 m.eq. per liter of plasma; the plasma chloride 104 m.eq. and the serum potassium 3.79 m.eq.

Case 5. Patient O.B., a white male teacher, 52 years old, in 1930 first noted loss of weight and strength, associated with dizziness on standing. A physician noted low blood pressure at this time. In 1934 physical examination revealed the blood pressure to be 88/58 and light pigmentation of the gums was found. The patient was treated with NaCl and a glycerol extract of adrenal cortex by mouth. This treatment resulted in considerable improvement and in the disappearance of the slight pigmentation of the gums. The patient was admitted to the University Hospital on 11-24-35 for special balance study to determine, if possible, whether the patient actually had early Addison's disease.

The physical examination at this time revealed nothing of note with the exception of the low blood pressure, 90/60.

Urinalysis gave normal findings. The hemoglobin was 14.6 gm. per 100 cc. The red blood cells numbered 4,839,000 and the white blood cells 6,800; there being 72 per cent polymorphonuclear, 20 per cent lymphocytes, 6 per cent eosinophils and 2 per cent monocytes. The non-protein nitrogen of the blood was 24 mg. per cent. The basal metabolic rate was —5 per cent. The concentration of blood plasma sodium was 135.6 m.eq., the plasma chloride 109.5 m.eq. and the serum potassium 5.18 m.eq.

OBSERVATIONS

Normal subjects. The results in 8 normal subjects are shown in figure 1. During the cortin period there occurred an increase in urine volume (11 per cent average). This was associated wth an increased excretion of sodium (10 per cent average), chloride (9 per cent average) and potassium (2 per cent average). It should be pointed out that the percentile changes in water, sodium and chloride excretion were nearly the same. Data obtained on 2 subjects who were studied during the same period as 8 normal subjects, but who did not receive cortin injections, showed a similar increase in urine volume as well as sodium and chloride excretion. (The average increases noted in these 2 subjects were 7 per cent in urine volume, 15 per cent sodium excretion, and 10 per cent chloride excretion.) The increased urine excretion was easily accounted for by the fall in temperature which occurred during the cortin period. The average mean temperature during this period was —1.6°C. as compared to 6.3°C. during the control period.

During the 5-day control period the 8 normal subjects showed an average daily negative nitrogen balance amounting to 2.95 gm. During the 5-day period of cortin injections the average negative nitrogen balance amounted to 4.67 gm. Approximately 4 per cent more nitrogen was excreted during the cortin experiment than during the control. This difference was more pronounced in the female subjects since increase in nitrogen excretion during the cortin

experiment amounted to only 1.2 per cent in the males as compared to 8 per cent for the females. Moreover, the 2 female control subjects showed variations in nitrogen excretion of about the same magnitude as those observed in the female subjects receiving cortin. Therefore the increased nitrogen excretion noted in these subjects cannot be attributed to cortin but was more likely due to some uncontrolled factor, perhaps the menstrual cycle (7).

Analyses of the blood plasma sodium and chloride, and serum potassium

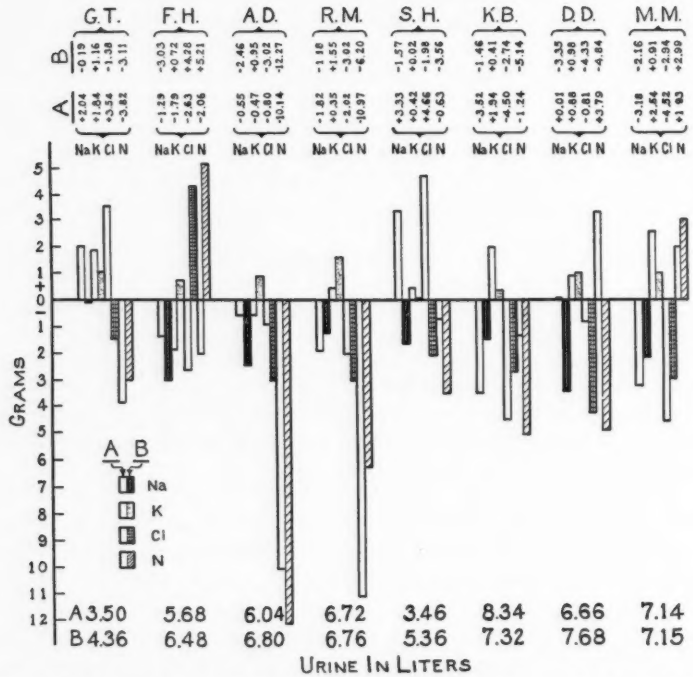

Fig. 1. Electrolyte and nitrogen balance in normal subjects treated with cortin. Totals for 5-day periods. *A*, Control Period. *B*, Cortin, 12 to 18 C.U. daily.

were made during the control period after the subjects had been on the diet for 4 days, and again 4 days after cortin injections had been instituted. The average values during the control period were as follows: sodium, 136.8 m.eq., chloride 110.5 m.eq. per liter of plasma and potassium 4.19 m.eq. per liter of serum. During the period of cortin injections the values were found to be 137.5 m.eq. for sodium, 112.6 m.eq. for chloride and 4.38 m.eq. for potassium. The percentage of cells as measured by the hematocrit changed from 45.8 per cent during the control period to 44.4 per cent during the cortin period. In the absence of blood volume determinations the changes in electrolyte concentration are too small to assume significance.

Patients with Addison's disease. Three patients with severe Addison's disease were studied. The results on patient C.B. have been incorporated in

figure 2. A negative sodium and chloride balance occurred during the control period. The excretion of sodium and chloride averaged 162.2 m.eq. and 138.8 m.eq. daily respectively as compared to an intake of 134.1 m.eq. of sodium and 129.2 m.eq. of chloride daily. No change in potassium balance was noted. During the control period the negative nitrogen balance averaged 1.43 gm. per day. This approximated closely the negative nitrogen balance (1.45 gm. daily) during the preliminary cortin period at which time the patient was receiving 12 C.U. of cortin daily. The average 24-hour urine volume during the control period amounted to 2,140 cc. as compared to an average of 1,736 cc. noted during the cortin period. The increased urine volume resulted in an average daily increase of 404 cc. during the 6 days of the control period. The body weight loss in this same period was 1.4 kg.

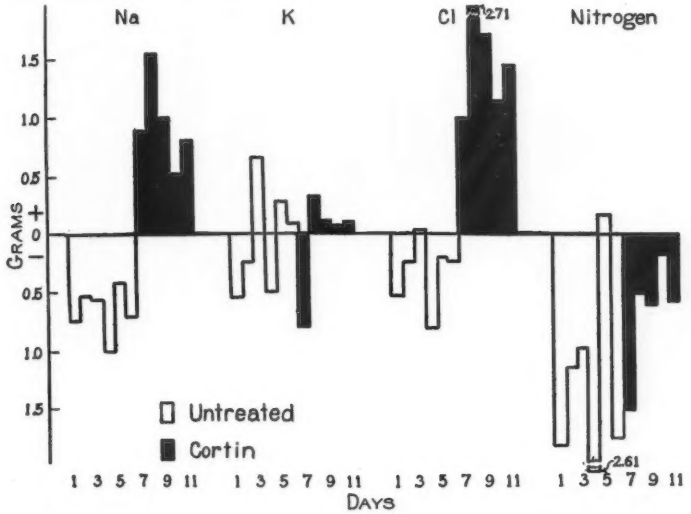

Fig. 2. Electrolyte and nitrogen balance in a patient (C.B.) with Addison's disease.

A retention of sodium and chloride occurred during the period of cortin treatment. An average of 41.1 m.eq. of sodium and 45.2 m.eq. of chloride daily were retained during the five days of treatment. Although little change (average 0.6 m.eq. daily) occurred in potassium balance during the cortin period, it should be noted that a marked excretion of potassium occurred on the first day of cortin treatment. The injection of 30 C.U. of cortin daily resulted in a 7 per cent reduction of the total nitrogen excretion. The negative nitrogen balance was reduced from 1.43 gm. per day during the control period to 0.69 gm. per day during the period of cortin treatment. The decrease in nitrogen loss was associated with a gain in body weight (1.3 kg.) and a reduction in the 24-hour urine volume (1,736 cc. as compared to 2,132 cc. during the control period). The alteration in body weight closely followed the change in urine volume.

On the first day of the control period, (3-31-36), analysis of the blood gave the following values: plasma sodium 129.0 m.eq., plasma chloride 102.1 m.eq. and serum potassium 5.23 m.eq. No blood determinations were made at the conclusion of this experiment. One month after cortin and sodium chloride therapy had been instituted the plasma sodium concentration of the blood was found to be 153.0 m.eq., the plasma chloride 108 m.eq. and the serum potassium 5.03 m.eq.

The results of the balance studies on patient R.R. have been incorporated in figure 3. There was a negative sodium and chloride balance during the

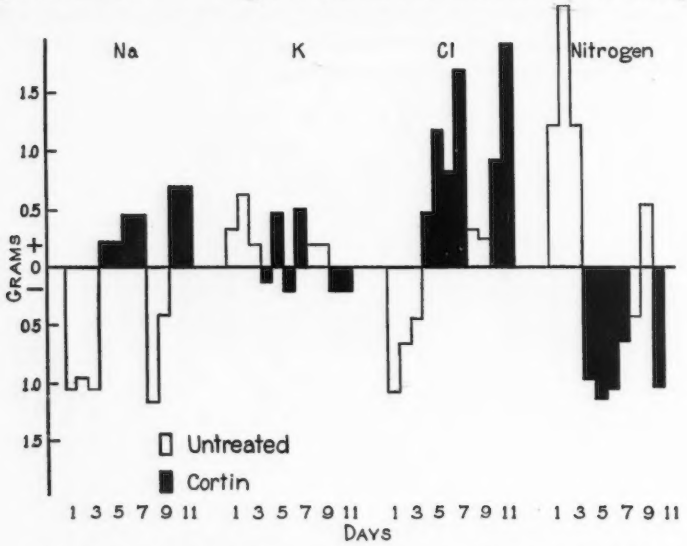

Fig. 3. Electrolyte and nitrogen balance in a patient (R.R.) with Addison's disease.

control periods. Cortin treatment resulted in a sodium and chloride retention and a potassium excretion. A positive nitrogen balance was shown on all but one day of the control periods, while a negative balance occurred during cortin treatment. It should be noted that the changes in total nitrogen excretion in patient C.B. were different from those observed in patient R.R., although retention of sodium and chloride and decreased urine volume associated with cortin treatment occurred in both. During the control period there was a positive balance in nitrogen in C.B. while during the corresponding period in R.R. the nitrogen balance was negative. The nitrogen balance during the cortin periods was negative in both patients.

It is possible for cortin to affect nitrogen metabolism in several ways. It might be expected to produce an increased excretion of urea and ammonia (8). These effects would tend to produce a negative nitrogen balance. On the other hand the increase in body weight which usually follows cortin therapy should be accompanied by a retention of nitrogen provided that such an increase results from the laying down of new tissue substance and not merely from the retention of water. There is also evidence that cortin therapy decreases the

creatinuria associated with untreated Addison's disease (unpublished data). These effects would tend to put the patient in a positive nitrogen balance. The end result obtained would depend on which of these effects predominated. Thus in patient C.B. whose body weight increased 1.3 kg. there occurred a progressive decrease in the negative nitrogen balance following the cortin treatment, whereas R.R. who gained only 0.2 kg. showed a marked negative nitrogen balance following cortin therapy while in control periods she was in a definite positive nitrogen balance. Additional experiments carried out on 2 patients with Addison's disease indicated that the administration of 8 to 10 gm. of NaCl by mouth daily, had no effect on the nitrogen balance.

During the experiment there was no significant alteration in the concentration of plasma sodium, chloride or serum potassium.

It seems pertinent to include the electrolyte balance study of patient I.B. obtained during a period of cortin treatment even though data for an untreated period are not available. During 6 days of cortin treatment a positive sodium and chloride balance was established. A total of 97.4 m.eq. of sodium and 66.1 m.eq. of chloride were retained. No change was noted in potassium balance. An increase in body weight (0.9 kg.) occurred during this period. On the first day of cortin treatment the plasma sodium was 145.2 m.eq., the plasma chloride 106 m.eq., and the serum potassium 4.27 m.eq. The rather high plasma sodium at this time may be accounted for in part by dehydration as the cell volume was found to be 45.6 per cent. Subsequent to 6 days of cortin treatment the blood values were as follows: plasma sodium 140.0 m.eq., plasma chloride 109.0 m.eq. and serum potassium 4.53 m.eq. The cell volume at this time amounted to 40.1 per cent. An increase in plasma volume of 297 cc. during this period (6 days) resulted in an increase of 31.8 m.eq. in the total plasma content of sodium, although the concentration of sodium per liter of plasma was actually decreased. Ten days of combined cortin and sodium chloride treatment were accompanied by an increase in plasma volume (498 cc.), a further reduction in the percentage of cells (35.7 per cent) and a slight increase in plasma sodium concentration (141.2 m.eq.). Calculated on the basis of the increased plasma volume this slight increase in plasma sodium concentration (1.2 m.eq.) actually represented an increase of 72.7 m.eq. in the total plasma content of sodium. This observation illustrates the inadequacy of plasma electrolyte determinations without a knowledge of blood volume in patients with Addison's disease.

Experience has impressed the authors with the value of beginning treatment early in Addison's disease. The difficulties involved in establishing a diagnosis at this time are well known. Determination of the plasma concentration of sodium and chloride is of little value as a significant alteration usually does not occur until the patient has reached a critical condition. Likewise it is doubtful whether changes in electrolyte balance would be of sufficient magnitude to be detected early in the disease. However, it seemed possible that the administration of cortin at this time might produce a significant retention of sodium and chloride.

To test the value of the effect of cortin on the sodium and chloride balance as a diagnostic procedure, 6 patients were selected who had in common some

of the signs and symptoms frequently associated with Addison's disease (asthenia, gastrointestinal disturbance, low blood pressure and in some instances an increase in pigmentation). Patient N.C. was known to have diabetes insipidus; patient M.W. had severe pulmonary fibrosis with emphysema; x-ray examination of patient H.M. indicated a duodenal ulcer; patient E.L. was experiencing menopausal changes; patient E.K. was shown to have a healed lesion in the upper lobe of the right lung which was presumed to have been tubercular in origin; patient O.B. had no evidence of disease other than low blood pressure, slight pigmentation of the gums, asthenia and weight loss.

In only 2 patients (E.K. and O.B.) did cortin injections produce a retention of sodium and chloride. In patient E.K. during a 5-day cortin-treated period (18 C.U. of cortin per day) 56.9 m.eq. of sodium and 122.0 m.eq. of chloride were retained in comparison to a 5-day control period during which no retention occurred. The weight gain during the treated period amounted to 0.5 kg. The blood pressure increased from 110 mm.Hg systolic and 70 mm. Hg diastolic to 128 mm.Hg systolic and 84 mm.Hg diastolic. Continued treatment during a period of 4 months resulted in a further increase in blood pressure to 170 mm.Hg systolic and 90 mm.Hg diastolic. The incompatibility of the symptoms of Addison's disease associated with a blood pressure of 110 mm.Hg systolic and 70 mm.Hg diastolic may be reconciled with a previously existing hypertension. Rowntree has noted several such cases (9).

In patient O.B. 4 days of cortin treatment (24 C.U. of injected cortin and 12 cc. of glycerol extract of adrenal cortex by mouth daily) resulted in a retention of 206 m.eq. of sodium and 145 m.eq. of chloride as compared to a control period during which a slight negative balance of sodium and chloride occurred. The blood pressure increased from 80 mm.Hg systolic and 58 mm. Hg diastolic before cortin treatment to 95 mm.Hg systolic and 72 mm.Hg diastolic after cortin treatment. The weight gain amounted to 1.5 kg. during this period. In both patients cortin injections were associated with an increased ability to perform work.

DISCUSSION

Daily cortin injections equivalent to the daily requirement of a severe case of Addison's disease seem to be without effect on the electrolyte balance in normal human beings. The increased urine volume, sodium, chloride and nitrogen excretion probably were not cortin effects in as much as 2 subjects not receiving cortin demonstrated similar changes. Change in the temperature of the environment may have been largely responsible. The negative effect of cortin on electrolyte balance in normal individuals makes the positive effect obtained in the patients assume greater significance. However, other studies conducted by the authors (10) in which 80 C.U. of cortin have been injected intravenously during a 5-hour experiment have demonstrated temporary but differential effect upon the renal excretion of electrolytes and water. Had larger doses of cortin been used in the balance studies of normal individuals the results might have been different.

In severe Addison's disease the experimental periods without treatment are necessarily shorter than is desirable for balance study. They demonstrate

the direction of change. The degree of negative sodium and chloride balance varies considerably in Addison's disease. It is most marked during a relapse or shortly after treatment is discontinued. Cortin treatment corrects the negative balance. Retention of sodium and chloride is accompanied by some decrease in urine volume. In patients C.B. and R.R. an increased potassium excretion occurred during the first 24 hours after treatment with cortin was begun. This effect appeared to be characteristic of cortin as both normal individuals and patients with Addison's disease have been shown to excrete large amounts of potassium in the urine during a 5-hour period in which 80 U. of cortin were given intravenously. The increase in the plasma concentration of sodium and chloride which occurs as a result of treatment (cortin and sodium chloride) is well known. However, frequent analysis of the blood during the early period of treatment may indicate that considerable clinical improvement may occur before the rise in plasma concentration of sodium and chloride. In one patient (I.B.) an actual decrease in plasma sodium concentration accompanied the institution of treatment. Blood volume studies have impressed the authors with the fact that a considerable retention of sodium may occur in the plasma as a result of an immediate increase in plasma volume. In such an instance a rise in the plasma concentration of sodium may be delayed. In spite of the critical condition of 2 of the patients with severe Addison's disease, the serum potassium value was never found to exceed 6 m.eq. However, this increase represents approximately the same percentile change as the decrease in plasma sodium.

A positive sodium and chloride balance was obtained during cortin treatment in 2 of the 6 patients (E.K. and O.B.) who presented some of the signs and symptoms common to Addison's disease. In both patients the sodium chloride of the diet was supplemented by an intravenous infusion of 500 cc. of 2 per cent NaCl during the control and treated period. The positive sodium and chloride balance associated with cortin injections was marked by a gain in body weight, an elevation of blood pressure and an improvement in appetite and ability to perform work. The subsequent response of these patients to continued therapy makes the diagnosis of early Addison's disease seem probable. A positive effect of cortin on sodium and chloride balance of a patient in whom Addison's disease is suspected makes the diagnosis more likely. Balance studies in female patients must take into account the marked variation in electrolyte excretion which may occur in some patients at or near catamenia as mentioned earlier.

Although the effect of cortin on electrolyte balance appears to be of definite value in the diagnosis of early Addison's disease, the facilities for such studies are not always available. Under such conditions a positive response to cortin characterized by gain in body weight, elevation of blood pressure, improved appetite and decreased asthenia may be interpreted as presumptive evidence of adrenal insufficiency. Care must be taken to control cortin treatment adequately so that improvement can be attributed to cortin alone. In so far as it is possible objective data only should constitute the basis for measuring such a response. There is no contraindication in the use of cortin as a diagnostic procedure.

SUMMARY

The authors have studied the effect of subcutaneous injections of cortin on the sodium, potassium and chloride balance of 8 normal subjects, 3 patients with severe Addison's disease, and 6 patients who presented some of the signs and symptoms common to this disease. The normal subjects showed no change which could not be accounted for by atmospheric conditions. The negative sodium and chloride balance in all of the patients with severe Addison's disease was changed to a positive balance by cortin. In 1 patient cortin treatment resulted in a marked negative potassium balance. In all 3 of the patients with severe Addison's disease a potassium diuresis occurred during the first 24 hours of treatment. The average urine volume of the 3 patients was greater during the period without treatment. Cortin treatment increased the plasma concentration of sodium and chloride in 2 of the patients. In 1 patient blood volume determinations demonstrated an increase in the plasma volume associated with an increase in total plasma sodium content which preceded a rise in plasma sodium concentration. In none of the patients did the serum potassium concentration exceed 6 m.eq. in spite of the critical condition of 2 of the patients. A rise in blood pressure and a gain in body weight accompanied the retention of sodium and chloride during the cortin period. The data indicate that the positive effect of cortin on the sodium and chloride balance is useful in the diagnosis of early Addison's disease.

We are indebted to the University Hospital for the use of its facilities in the conduct of this investigation. We are especially indebted to Dr. Carl Moore for the blood volume studies.

REFERENCES

1. Atchley, D. W., R. F. Loeb, D. W. Richards, Jr., E. M. Benedict, and M. E. Driscoll: J. Clin. Investigation **12:** 297. 1933.
2. Butler, A. M., and E. J. Tuthill: J. Biol. Chem. **93:** 171. 1931.
3. Saifer, A., and M. Kornblum. J. Biol. Chem. **112:** 117. 1935.
4. Peters, J. P., and D. D. VanSlyke: Quantitative Clinical Chemistry **2:** 829. 1932. Williams and Wilkins Co., Baltimore.
5. Hubbard, R. S.: Personal Communication.
6. Hubbard, R. S.: J. Biol. Chem. **100:** 557. 1933.
7. Pucher, G. W., F. R. Griffith, Jr., Katharine A. Brownell, Jennie D. Klein, and Mable E. Carmer: J. Nutrition **7:** 169. 1934.
8. Stahl, J., D. W. Atchley, and R. F. Loeb: J. Clin. Investigation **15:** 41. 1936.
9. Rowntree, L. G., and A. M. Snell: Mayo Clinic Monographs, A Clinical Study of Addison's Disease, p. 166, W. B. Saunders Co., Philadelphia and London, 1931.
10. Thorn, G. W., Helen R. Garbutt, F. A. Hitchcock, and F. A. Hartman: Endocrinology **21:** 213. 1937.

THE EFFECT OF CORTIN UPON THE RENAL EXCRETION OF SODIUM, POTASSIUM, CHLORIDE, INORGANIC PHOSPHOROUS AND TOTAL NITROGEN IN NORMAL SUBJECTS AND IN PATIENTS WITH ADDISON'S DISEASE[1]

GEORGE W. THORN, HELEN R. GARBUTT, FRED A. HITCHCOCK AND FRANK A. HARTMAN

From the Department of Physiology, The Ohio State University
COLUMBUS, OHIO

In another investigation (1) we have shown that amounts of cortin sufficient to maintain patients with severe Addison's disease have little or no effect on the electrolyte and nitrogen balance of normal human beings. There was still the possibility that very large doses of cortin might effect the balance in normal individuals. Cost makes the use of massive doses almost impossible in balance studies. However, when large doses are used the effect on the excretion of sodium, potassium and chloride is so prompt that balance studies are not required. The immediate effect of large injections of cortin on the renal excretion of sodium, potassium, chloride and total nitrogen is reported in this paper.

METHODS

The subjects ingested no food after 6 o'clock on the evening preceding an experiment. They remained at rest throughout the entire period. At 5 A.M. the night urine was voided and 100 cc. of water was drunk. At the end of each subsequent hour the urine was collected and 100 cc. of water was ingested, the last collection being at 12:00 noon. The water ingested produced enough urine for analysis. At 7:00 A.M., 8:00 A.M., 9:00 A.M. and 10:00 A.M., 25, 25, 15 and 15 cat units of cortin respectively, in 5 cc. of normal saline solution were injected intravenously. As a control, duplicate experiments were conducted on the same individuals using injections of 5 cc. of normal saline solution. At least 48 hours intervened between the control experiment and the cortin experiment. Although the experiments were conducted during the fasting state an attempt was made to control the conditions of the experiment by providing both the normal subjects and the patients with a diet of constant composition. The diet of the patients was weighed and analyzed. The daily amount of the food ingested by the 4 normal subjects was carefully approximated, a similar monotonous diet being provided for all 4 subjects during the entire period. In all instances the added sodium chloride was weighed.

The urine specimens were collected in stoppered bottles and then placed

[1] Aided by a grant from The Rockefeller Foundation.

in a cold room maintained at 4°C. Phosphate analyses were made within 3 hours from the time the urine specimens were collected. Toluene was then added to the urine. Venous blood samples were drawn with as little stasis as

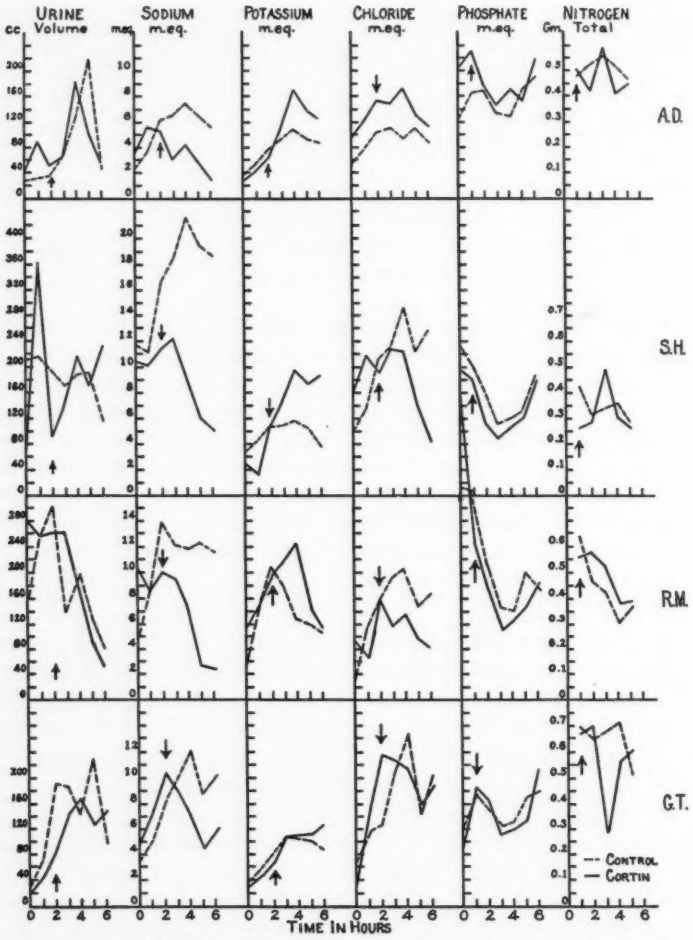

Fig. 1. The influence of cortin on renal excretion in normal subjects. Each subject received 5 cc. of 0.9 per cent NaCl intravenously at 7:00 A.M., 8:00 A.M., 9:00 A.M. and 10:00 A.M. during both experiments "A" and "B." During experiment "B" the 7:00 A.M. and 8:00 A.M. injections each contained 25 C.U. of cortin. The 9:00 A.M. and 10:00 A.M. injections each contained 15 C.U. of cortin. Phosphate values are in millimols instead of m. eg. as shown.

possible at 7:00 A.M. and at 12:00 noon. The blood samples were centrifuged immediately at 3,000 r.p.m. for 50 minutes and the plasma used for analysis. Heparin was used as an anticoagulant.

The analytical methods employed were as follows: Sodium was determined according to the method of Butler and Tuthill (2). The plasma chloride

determinations are made according to the adsorption indicator method as described by Saifer and Kornblum (3). The open Carius method with a modification of the Volhard-Harvey titration was used for analyzing the urine and diet chloride (4). Potassium determinations of the blood serum, urine and diet were made according to the method of Hubbard (5, 6). The inorganic phosphate of the urine was analyzed according to the method of Fiske and Subbarow (7). Total nitrogen was determined by the macro Kjeldahl method.

RESULTS

The results of the experiments on normal subjects are shown graphically in figure 1. A comparison of the urine volume in cortin and control experiments shows considerable variation in the hourly values but the total for the 5-hour period corresponds well in all but 1 subject. In this subject (G.T.) the volume excreted during the cortin experiment was reduced 20.5 per cent.

TABLE 1. THE AVERAGE EXCRETION OF SODIUM, POTASSIUM, CHLORIDE, PHOSPHATE AND TOTAL NITROGEN IN 4 NORMAL SUBJECTS FOR 5-HOUR PERIODS AS AFFECTED BY CORTIN

	Sodium m.eq.	Potassium m.eq.	Chloride m.eq.	Phosphate m.eq.	Total Nitrogen gm.	Urine Volume cc.
Control period	58.1	27.4	44.4	39.2	2.75	764
Cortin period	33.6	35.5	40.1	37.4	2.63	708
Per cent change	−42	+29.5	−9.6	−4.6	−4.36	−7.3

Sodium was retained in all subjects after the injection of cortin. Retention was very evident by the third hour, becoming more marked later. All 4 subjects excreted an increased amount of potassium during the cortin experiment, while chloride and inorganic phosphate were reduced in 2 subjects (S.H. and R.M.), little modified in a third (G.T.) and increased in the fourth (A.D.). The injection of cortin seems to have little or no effect on the excretion of total nitrogen although in 3 subjects there was a marked increase in nitrogen excretion during at least 1 hour of the cortin experiment. The average changes in the 4 subjects are given in table 1. Two additional control experiments were conducted. In one group of experiments 3 subjects were injected with a cortin solution which had been kept at a temperature of 100°C. for periods ranging from 1 to 4 hours. These experiments demonstrated an effect on the sodium, potassium and chloride excretion which was similar to that noted in the unheated cortin solution experiment, but less marked.

The cortin solution used in these experiments contained epinephrin in the proportion of 1:200,000, according to the Folin, Cannon and Denis colorimetric method. The actual concentration was probably much less than this since tests in this laboratory have shown that the colorimetric method gives results 5 to 10 times higher than values obtained by the intestinal strip method. In order to determine the possible effects of the small amount of epinephrin contained in the cortin solution a control test was run on 1 subject (G.T.) in which 5 cc. of a solution of 1:200,000 adrenalin chloride in 0.9 per cent sodium chloride was injected intravenously every hour for 4 hours. This

subject was very sensitive to adrenalin. Each of the injections resulted in a marked but transient adrenalin reaction characterized by trembling, tachycardia and sweating. None of these reactions occurred after cortin injections. During the adrenalin experiment the urine volume was greatly reduced being less than 60 per cent of that noted in the control. The sodium excretion was

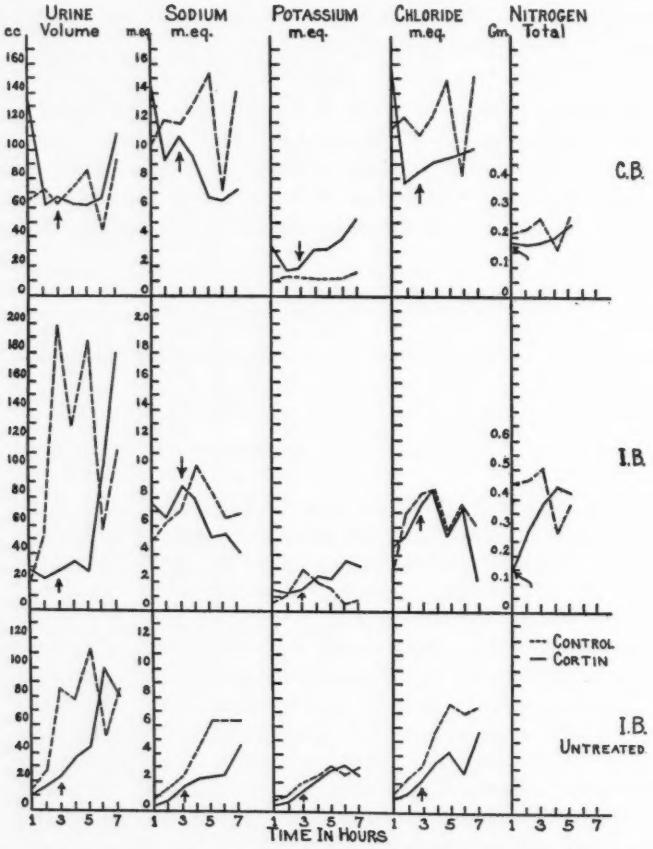

Fig. 2. The influence of cortin on renal excretion in patients with Addison's disease. Each subject received 5 cc. of 0.9 per cent NaCl intravenously at 7:00 A.M., 8:00 A.M., 9:00 A.M. and 10:00 A.M. during both experiments "A" and "B." During experiment "B" the 7:00 A.M. and 8:00 A.M. injections each contained 25 C.U. of cortin. The 9:00 A.M. and 10:00 A.M. injections each contained 15 C.U. of cortin. "I.B. untreated" without treatment for 2 weeks.

reduced to 68 per cent, the chloride to 70 per cent and the potassium to 46 per cent of that noted during the control experiment. It is impossible to estimate what percentage of the reduced urine volume and constituents represent an effect of epinephrin upon renal excretion and what percentage of the reduction in urine volume and electrolyte excretion may have been due to the losses occurring in the sweat. It should be pointed out that in this experiment

reduced urine volume was associated with a marked reduction in the excretion of all of the electrolytes measured, there being no differential excretion such as was found in the cortin experiments.

Analyses of the plasma electrolytes were made at 7:00 A.M. and again at 12:00 noon in all subjects. No significant variations occurred in the concentration of plasma sodium and chloride or serum potassium during the cortin experiment as compared to the control experiment.

The data obtained from a study of 2 patients with Addison's disease (C.B. and I.B.) are presented in figure 2. During these studies the patients were maintained in good condition by the daily administration of 24 cat units of injected cortin and 8 grams of sodium chloride by mouth except in a second experiment on I.B. during which no specific therapy was administered. In both the control and cortin experiments, routine treatment was discontinued at 4 o'clock on the afternoon of the day preceding the special test. In this manner it was possible to study the immediate effect of an intravenous injection of cortin.

The results in the 2 patients with Addison's disease were similar to those obtained in the normal subjects except that the urine volume was decreased considerably after cortin injections in patient I.B. There was marked sodium retention after cortin in both patients while large amounts of potassium were excreted. In I.B. there was also an increase in the excretion of inorganic phosphate (27 per cent). No determinations of inorganic phosphates were made for C.B. The chloride excretion was somewhat reduced in both patients but there was no significant change in nitrogen excretion.

In 1 patient (I.B.) the immediate effect of cortin injections was studied 24 days after maintenance cortin and sodium chloride had been discontinued. During this period the patient had lost weight and strength and had been confined to bed. There was evidence of marked dehydration. For 8 days previous to the experiment this patient was maintained on a constant weighed diet. The results of cortin injections were similar to those obtained in experiment I, except that the potassium excretion was little affected during the five hour period.

DISCUSSION

In another investigation (1), eight normal subjects were studied for a period of 17 days while being maintained on a constant diet and liquid intake. At the end of a 7-day control period 6 cat units of cortin were injected subcutaneously 2 or 3 times daily for a period of 5 days. A number of observations were made including plasma electrolyte determinations and the total electrolyte balance. From an analysis of the total 24-hour urine specimen no remarkable alteration in the excretion of sodium, potassium, chloride and total nitrogen could be detected. It seemed more likely that an effect of cortin might be noted if the renal excretion was studied within a short time after the cortin injections, particularly if larger quantities of cortin were used.

The present experiments on normal subjects demonstrate the effectiveness of larger amounts of cortin. The retention of sodium was not associated with any significant alteration in the plasma concentration of this electrolyte. The

failure of the plasma to reflect the decreased sodium excretion would suggest either a tissue retention of this substance or an increase in plasma volume (8). Studies on patients with Addison's disease have demonstrated that the retention of sodium and chloride as a result of cortin treatment is associated with an increased plasma volume (to be published), which however does not account for all of the sodium and chloride retained.

No significant variation in the serum concentration of potassium occurred in either the control or cortin experiments in the 4 normal subjects in spite of the renal excretion of large quantities of potassium (23 to 45 m.eq.). This potassium loss was equivalent to the total potassium content of from 6 to 10 liters of serum.

The amount of potassium and sodium excreted by patient I.B. (fig. 2) was small in comparison to that excreted by the 4 normal subjects and patient C.B. This patient had been without treatment for 2 weeks and showed evidence of marked dehydration and inanition. Experiments performed, before treatment was instituted, demonstrated no additional loss of potassium associated with the first cortin injections, during the 5-hour period, although a sodium retention was noted. This is the only experiment in which there was no potassium loss accompanying the retention of sodium.

In this report cortin injections in 2 of the 4 normal subjects and in all of the patients with Addison's disease resulted in some retention of chloride. This was not as marked as the sodium retention. The alteration in the excretion of chloride associated with adrenal insufficiency would appear to be secondary to the alteration in base.

The phosphate excretion in the 4 normal subjects was not significantly altered by the cortin injections. In subjects S.H. and R.M. there occurred a 13 per cent and a 14 per cent reduction respectively in the amount of inorganic phosphate excreted during the cortin experiment. Subjects A.D. and G.T. excreted 8 per cent and 1 per cent more phosphate respectively during the cortin experiment. It appears that cortin has little immediate effect upon the renal excretion of phosphate in normal subjects.

The results obtained on the total nitrogen excretion would seem to indicate that cortin has no significant effect on the excretion of this substance. Apparently when nitrogen excretion is normal as it was even with the patients having Addison's disease, the injection of cortin is without effect. It should be pointed out however, that although total nitrogen was unaffected it is entirely possible that there was an effect on the nitrogen partition which would not have been detected by our procedure (9).

A differential effect of cortin on the renal excretion of sodium and potassium has been observed in normal subjects as well as in patients with Addison's disease. Previous attempts to show a positive effect of cortin in normal subjects have met with little success. This has been due to inadequate dosage. The present experiments demonstrate that large intravenous injections of cortin produce a prompt effect on the kidney. Eighty cat units in 4 hours is 3 or 4 times the amount required by a patient with severe Addison's disease for 24 hours. If this dosage were continued throughout 24 hours the subject

would receive 18 to 24 times the requirement for a severe case of Addison's disease.

SUMMARY

The effect of cortin on the renal excretion of sodium, potassium, chloride, inorganic phosphates and total nitrogen was studied in 4 normal subjects and in 2 patients with Addison's disease. Both subjects and patients were maintained on a constant diet and liquid intake. Hourly urine specimens were collected during the fasting state with the subject at rest. Cortin was injected intravenously each hour for 4 hours, a total of 80 cat units being injected in each normal subject and in each patient. Sodium chloride (0.9 per cent), heated cortin solution and a dilute adrenalin solution were used as control injections.

Cortin injections were associated with a marked reduction in the hourly excretion of sodium in normal subjects as well as in patients with Addison's disease. Potassium excretion was greatly increased during the cortin injections in both normal subjects and patients. Some reduction in chloride excretion accompanied the cortin injections in 2 normal subjects and in both patients with Addison's disease. There was no significant effect on the excretion of inorganic phosphates or total nitrogen.

These results demonstrate that it is possible to obtain measurable effects of cortin in normal subjects if sufficiently large amounts of the hormone are used.

We wish to express our appreciation to Doctor A. J. Derbyshire, Messrs. S. Horvath and R. Miller and the staff of the University Hospital for their assistance in this investigation.

REFERENCES

1. Thorn, G. W., Helen R. Garbutt, F. A. Hitchcock, and F. A. Hartman: Endocrinology **21:** 202. 1937.
2. Butler, A. M., and E. J. Tuthill: J. Biol. Chem. **93:** 171. 1931.
3. Saifer, A., and M. Kornblum: J. Biol. Chem. **112:** 117. 1935.
4. Peters, J. P., and D. D. VanSlyke: Quantitative Clinical Chemistry, **2:** 829. 1932. Williams and Wilkins Co., Baltimore.
5. Hubbard, R. S.: Personal Communication.
6. Hubbard, R. S.: J. Biol. Chem. **100:** 557. 1933.
7. Fiske, C. H., and Y. Subbarow: J. Biol. Chem. **66:** 375. 1925.
8. Harrop, G. A.: Bull. Johns Hopkins Hosp. **59:** 33. 1936.
9. Stahl, J., D. W. Atchley, and R. F. Loeb: J. Clin. Investigation. **15:** 41. 1936.

THE TREATMENT OF UNDESCENDED TESTES WITH ANTERIOR PITUITARY-LIKE SUBSTANCE[1]

W. O. THOMPSON, A. D. BEVAN, N. J. HECKEL, E. R. McCARTHY, AND P. K. THOMPSON

From the Departments of Medicine and Surgery, Rush Medical College of the University of Chicago, and the Presbyterian and Cook County Hospitals

CHICAGO, ILLINOIS

Clinical findings, beginning with those of Cushing and his co-workers (1) and of Aschner (2) about 25 years ago, and the more recent experimental results of Zondek (3), Smith (4), Smith and Engle (5), Evans and Long (6), Moore and co-workers (7), in animals, furnish clear cut evidence of the effect of the anterior lobe of the pituitary on the growth and function of the gonads. While extensive observations on animals were being carried out by various investigators on the effects of the pituitary, Aschheim and Zondek (8) noted the presence in the urine of pregnant women of a substance that stimulated the sex glands. Shortly afterward, the production of genital growth and testicular descent with this material was reported by Schapiro (9) in a large percentage of boys and young men with hypogenitalism and cryptorchidism. Since then several reports have appeared, most of which give high percentages of successful results. To some surgeons familiar with the finding at operation of anatomical factors making descent difficult or impossible, many of these reports appeared enthusiastic. It therefore seemed desirable to make a critical study of the problem by a group of observers, one of whom should be a surgeon of wide and extensive experience in this field (10, 11, 12).

Embryological considerations. The testis appears about the 5th week as the genital ridge or mass. There are differences of opinion concerning some of the details of its development. The consensus among embryologists is that the cells of the distal portion of the mass proliferate, while those of the proximal end atrophy; at the same time the fetus grows so that the testis appears to descend to the level of the inguinal ring, which is being prepared for it. By the 4th month, the testis lies just inside the internal inguinal ring; by the 7th month, at the lower end of the canal; and during the last month of intra-uterine life it descends into the scrotum. The pouch of peritoneum which later becomes the tunica vaginalis precedes the testis into the scrotum. The gubernaculum is attached very early to the genital ridge as it develops downward and, in the beginning, has attachments to the abdominal muscles, the root of the penis, Scarpa's triangle, the base of the scrotum and the perineum. At operation, undescended testes are usually found just inside the internal ring, in the inguinal canal, or deflected from the external ring in various abnormal positions, usually those in which fibres of the gubernaculum are found early in its development. In addition to various anomalies of development, it is usually found that

[1] Read before the 20th Annual Scientific Session of the Association for the Study of Internal Secretions, Kansas City, May 12, 1936.

descent of the testis is prevented by the shortness of the various structures to which it and the cord are attached, namely, the peritoneal process, the transversalis fascia, the cremasteric muscle and fascia and the intercolumnar fascia.

It is of interest that the primates are the only species in which the testes normally descend either before or at birth. In such animals as the guinea pig, the rat, the ground squirrel, and the rabbit, descent occurs either at puberty or during the breeding periods (13).

TABLE 1. ANALYSIS OF PUBLISHED REPORTS ON TREATMENT OF UNDESCENDED TESTES WITH APL SUBSTANCE, WITH AND WITHOUT OTHER THERAPY

Author	No. of patients	Av. age, years	Total no. undescended testes	Total no. descended	Per cent descended	Type				Location							
						Bilateral		Unilateral		Intra-abd.		Inguinal		Upper scrotum		Not stated	
						No. before treatment	No. descended	No. before treatment	No. descended	No. before treatment	No. descended	No. before treatment	No. descended	No. before treatment	Number descended	No. before treatment	No. descended
Aberle & Jenkins (14)	4	7	5	2	40	2	0	3	2	1	0	4	2				
Brosius (15	10	3 to 38	14	8	57	8	4	6	4	5	4	4	3	3	1	2	0
Cohn (16)	5	9.5	6	5	84	2	2	4	3	2	1	3	3	1	1		
Dorff (17)	7	8.5	10	7	70	6	4	4	3	5	4	5	3				
Goldman & Stern (18)	2	13.5	2	1	50			2	1							2	1
Huberman & Israeloff (13)	5	11	7	5	71	4	4	3	1	4	4	3	1				
Kuhnstadter & Robins (19)	3	10	5	5	100	4	4	1	1							5	5
Kuhnstadter (20)	13	—	23	19	82	20	18	3	1							23	19
Priestley & Cohen (21)	2	12	4	1	25	4	1					4	1				
Rubinstein (22)	1	10.5	2	1 1p	artial 87	2	1			2							
Sexton (23)	6	12.5	9	7	78	6	4	3	3	2	2	4	2			3	3
Sippe (24)	1	10	2	2	100	2	2			1	1	1	1				
Spence & Scowen (25)	33	4 to 26	45	29	64	24	16	21	13					2	2	43	27
Webster (26)	11	11	14	13	93	6	5	8	8	7	7	7	6				
Totals	103		148	106	72	90	66	58	40	29	25	35	22	6	4	78	55
Schapiro (9)	13																
	116																

Summary of published results. A summary of the results of other observers is recorded in table 1. There were 148 undescended testes in 103 patients. The condition was bilateral in 45 patients and unilateral in 58. Schapiro (9) does not state in how many instances the condition was bilateral and therefore we have not been able to use his patients in our percentages. Of the total number of undescended testes, descent occurred in 106 cases, or 72 per cent. These successful results may be divided according to the original position as follows.

Intra-abdominal: descent reported in 25 of 29 cases.....83 per cent.
Inguinal: descent reported in 22 of 35 cases.....61 per cent.
In upper scrotum: descent reported in 2 of 2 cases.....100 per cent.
Unstated location: descent reported in 55 of 78 cases.....70 per cent

TABLE 2.

Patient, date and age	Description of patient	Pre-operative dose—APL substance R.U.	No. of undescended testes	No. that descended	Location				Effect of APL substance on genitalia and general condition
					Intra-abdominal		Inguinal		
					Before treatment	No. descended	Before treatment	No. descended	
J.P. 8/15/35 1.5 yr.	Well developed, alert. Rt. testis not palpable.	3,925 8 mo.	1	0	1	0			Left testis increased from $\frac{1}{4}''\times\frac{1}{4}''$ to $1\frac{1}{4}''\times\frac{1}{4}''$. Penis unchanged. Right testis palpable in upper part of canal, on standing.
F.S. 8/15/35 6 yr.	Well developed. Left congenital hernia. Rt. testis about at level of internal ring on standing.	6,300 8 mo.	1	0	1	0			Left testis increased from $\frac{1}{2}''\times\frac{1}{2}''$ to $1''\times 9/_{16}''$. Right testis in canal and can be pushed back into abdomen. Penis increased from $2''\times\frac{1}{2}''$ to $2\frac{1}{2}''\times\frac{1}{2}''$. More erections. More active and masculine.
R.B. 8/ 1/35 8 yr.	Moderately well developed; short for age. Masculine. Rt. testis not palpable.	8,000 3.5 mo.	1	0	1	0			Left testis increased from $\frac{1}{2}''\times\frac{1}{2}''$ to $1\frac{1}{2}''\times\frac{1}{2}''$. Penis increased from $1\frac{1}{2}''\times\frac{1}{2}''$ to $2\frac{1}{2}''\times\frac{1}{2}''$. More erections. More lively, better appetite.
M.A. 7/31/35 12 yr.	Well nourished. Left inguinal hernia; left testis not palpable.[1]	8,175 4.8 mo.	1	0	1	0			Right testis increased from $\frac{3}{4}''\times 1''$ to $\frac{3}{4}''\times 1\frac{1}{2}''$. Left testis could be felt high up in canal. Penis changed from $1\frac{1}{2}''\times\frac{1}{2}''$ to $2''\times\frac{1}{2}''$. More erections.
R.P. 12/ 9/35 15 yr.	Fröhlich. Neither testis palpable.	9,370 4.8 mo.	2	0	2	0			Penis increased from $1''\times 1\frac{1}{2}''$ to $1\frac{1}{2}''\times 1\frac{1}{2}''$. Some increase in pubic hair.
R.E. 8/19/35 16 yr.	Short, underweight; fem. hair distr. Bilateral inguinal hernia. Rt. testis palpable upper end canal; left testis not palpable.	2,580 1 mo.	2	0	1	0	1	0	No change noted except increased masturbation.
C.J. 2/10/36 16 yr.	Broad hips, narrow chest, effeminate. Rt. testis not palpable.	4,840 2.5 mo.	1	0	1	0			No change in left testis. Penis increased from $1\frac{1}{2}''\times 3\frac{1}{2}''$ to $1\frac{1}{2}''\times 4''$.
A.V. 9/26/34 5 yr.	Well developed. Hypospadias. Left testis of migratory type.	3,385 in 3 courses, 18 mo.	1	1			1	1	Measurements not recorded. Left testis descended within 2 weeks after starting last course.
A.W. 8/ 1/35 7 yr.	Mod. well developed, somewhat effeminate. Rt. testis in middle inguinal canal; can be pushed to upper end of scrotum. Hydrocele of left spermatic cord.	12,000 8.3 mo.	1	0			1	0	Left testis increased from $1''\times\frac{1}{2}''$ to $1\frac{1}{2}''\times\frac{1}{2}''$. Penis increased from $1\frac{1}{2}''\times\frac{1}{2}''$ to $2\frac{1}{2}''\times\frac{1}{2}''$. More erections. More masculine.
C.D. 8/ 9/35 7 yr.	Well developed. Masculine. Rt. inguinal hernia. Rt. testis in upper part of canal, can be pushed to upper end of scrotum.	5,440 2 mo.	1	0			1	0	Left testis increased from $\frac{1}{2}''\times\frac{1}{2}''$ to $1\frac{1}{2}''\times\frac{1}{2}''$. Penis increased from $1\frac{1}{2}''\times\frac{1}{2}''$ to $1\frac{1}{2}''\times 13/_{16}''$. More erections. More belligerent and masculine. (Fig. 3).
A.C. 12/ 4/35 8 yr.	Mod. well developed; epilepsy; mentally retarded. Left inguinal hernia. Left testis in lower part of canal, can be pushed to upper end of scrotum.	6,740 5 mo.	1	0			1	0	Right testis showed no change. Measurements $1\frac{1}{2}''\times\frac{1}{2}''$. Penis increased from $2\frac{1}{2}''\times\frac{1}{2}''$ to $2\frac{1}{2}''\times\frac{1}{2}''$. More lively. Convulsions less frequent. (Fig. 3.)
T.G. 10/28/34 8 yr.	Active, effeminate. Left inguinal hernia. Left testis high in inguinal canal; can be pushed halfway to scrotum.	4,590 4.5 mo.	1	0			1	0	No change in right testis or penis.
O.B. 8/21/35 9 yr.	Slim, effeminate. Probably small right inguinal hernia. Both testes in canal and can be pushed to upper end of scrotum.	17,260 8 mo.	2	1			2	1	Penis increased from $1''\times\frac{1}{2}''$ to $1\frac{1}{2}''\times\frac{1}{2}''$. More alert and active. Right testis descended within 1 month.

TABLE 2. (*Continued*)

Patient, date and age	Description of patient	Pre-operative dose—APL substance R.U.	No. of undescended testes	No. that descended	Location				Effect of APL substance on genitalia and general condition
					Intra-abdominal		Inguinal		
					Before treatment	No. descended	Before treatment	No. descended	
R.B. 9/13/34 9 yr.	Bilateral club feet. Mentally retarded. Rt. testicle of migratory type.	940 2 wks.	1	1			1	1	Left testis increased from $1''\times{}^{7}/_{16}''$ to $1\frac{1}{4}''\times\frac{3}{4}''$. The right testis which came down in Sept. 1934 within 2 weeks after treatment, was found to be up in the canal again in Feb. 1936. (Fig. 1).
I.K. 5/ 6/35 11 yr.	Well developed. Right testis high in canal, can be pulled down to upper end of scrotum.	12,900 8 mo.	1	0			1	0	Right testis increased from $\frac{1}{8}''\times\frac{1}{4}''$ to $\frac{3}{4}''\times1\frac{1}{2}''$. Left testis increased from $\frac{1}{2}''\times1''$ to $\frac{1}{2}''\times1\frac{1}{2}''$. Penis increased from 1" length to $1\frac{1}{2}''$. (Fig. 2).
P.L. 7/18/35 11 yr.	Masculine. Left testis descends halfway into canal on standing.[1]	7,740 5.5. mo.	1	0			1	0	Penis increased from $2''\times{}^{7}/_{16}''$ to $2\frac{1}{2}''\times\frac{1}{2}''$. More erections. Increased number of pubic hairs.
F.N. 8/10/35 12 yr.	Masculine. Left testis can be pushed to middle of inguinal canal but recedes into abdomen when pressure is released.[2]	625 2 wks.	1	1			1	1	Penis $2\frac{1}{4}''\times\frac{1}{2}''$ before treatment. $2\frac{1}{4}''\times{}^{13}/_{16}''$ after treatment. More lively. Left testis descended within 2 weeks.
J.D.P. 1/18/36 17 yr.	Well developed. Masculine type. Rt. testis in canal; cannot be moved down to ext. ring.	5,200[3] 1 mo.	1	0			1	0	Comparative measurements not made.

[1] Right orchidopexy 2 years before.
[2] Right orchidopexy 2 weeks before.
[3] This patient received antuitrin S. instead of follutein.

The percentage of successful results in the intra-abdominal cases may be somewhat too high because of the lower percentage in the large number of cases in which the location was not stated. It would be highly desirable to know how many of the testes were of the migratory type, that is, those which move back and forth from the canal to the scrotum. It has been repeatedly demonstrated that this type of testis tends to remain permanently in the scrotum after puberty and its variable position may lead to the recording of false results. As an example, we had one surgical resident who became so enthusiastic about the treatment that he was led to mistake the results of manipulation for those of the anterior pituitary-like substance. According to McKenna and Ewert (27), this material is of value only in the migratory type. When descent occurs, it commonly does so within the first 3 weeks of treatment (25). According to Sexton (23) results are most favorable in early adolescence.

MATERIAL USED

We used follutein of E. R. Squibb and Sons. This material originally contained 125 R.U. per cc. but has recently been changed to contain 100 R.U. per cc. It is a sterile glycerin solution of the anterior pituitary-like sex factor found in pregnancy urine. Other names for this substance are antuitrin-S, urinary hebin, prolan, prolan A and B, rho 1 and 2 präehormon, APL factor, gynantrin and antophysin. While the effect of this material on the gonads in many ways resembles that of the anterior lobe of the pituitary, there are many points of difference, notably, its apparent failure to affect the ovary in the

absence of the pituitary. There is considerable discussion about the site of its action, some observers claiming that it affects only the interstitial cells of the testis and has no effect on the seminiferous tubules, while others claim it stimulates the production of spermatozoa (28, 29, 30).

RESULTS

Our findings are summarized in table 2. It may be seen that descent occurred in only 4 of 21 undescended testes in 18 patients. However, 3 of the patients had had an orchidopexy before treatment was started, so that originally the condition was bilateral in 6 of the 18 patients. In the 4 patients showing descent with treatment, the testis was in the inguinal canal.

An analysis of the successful cases is of interest. In one case there was some question concerning the location. Unfortunately, the first examination was made with the patient in the recumbent position and the testis could not be felt. This led to the conclusion that it was confined within the abdominal cavity. However at a second examination made 1 week later with the patient in the erect position, the testis was felt high up in the canal. Testes which cannot be palpated in the recumbent position can often be felt when the upright position is assumed. In another case (R.B., fig. 1) there is some question concerning descent. After the patient had received 940 U. of follutein in 2 weeks, the testis remained in the scrotum for a time, only to ascend into the canal when treatment was stopped. The testis could be pushed into the upper end of the scrotum before treatment was started. At the present time it is still in the canal but the patient will not report for further treatment. Of the other 2 successful cases, the testis could be pushed down to the lower end of the canal in one and to the upper end of the scrotum in the other. Thus in 3 of our 4 successful results, the testes were of the type that commonly descends at puberty. In one of the patients (O.B.) in whom a successful result was reported, the condition was bilateral and descent occurred on only one side. The findings at operation in several of the unsuccessful cases would suggest that anatomical factors were responsible for failure of descent. In most of the 18 patients an increase in the size of the external genitalia occurred.

Two possible criticisms of our results are as follows. *A)*. The material is water-soluble and rapidly excreted and might have been more effective if injected daily instead of every other day. *B)*. Perhaps the treatment should have been carried out for a longer time and in larger doses. Our patients received as large doses of the APL substance as those of some authors reporting a much higher percentage of successful results. The fact that descent occurred within 1 month in all 4 of the successful cases, and that the external genitalia increased in size in practically all cases, together with the findings at operation, suggest that our treatment was probably adequate. While the number of instances in which descent occurred is small, the increase in size and vascularity of the genital organs appeared to make the subsequent operative procedures less difficult. For this reason it would appear desirable, in the present state of our knowledge, to treat all patients from 4 to 6 months with anterior pituitary-like substance and to carry out operative procedures in those in whom

descent fails to occur. The ideal age for the treatment has not been determined. The material tends to induce changes which simulate premature puberty in the younger boys and the advisability of such a procedure should be carefully considered.

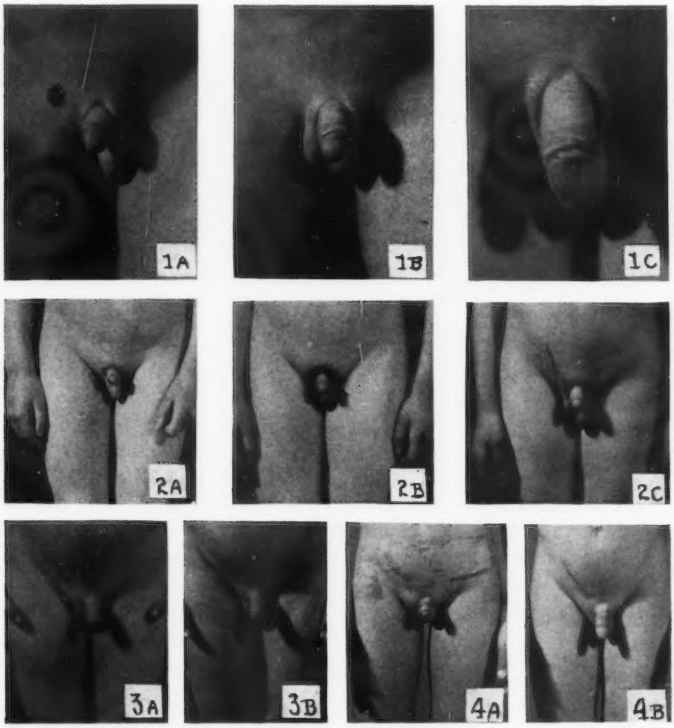

Fig. 1. R.B., aged 9 yr. Right undescended testis of migratory type. *A).* 8/13/34. Before treatment. *B).* 10/2/34. In scrotum during short course of treatment with follutein. *C).* 2/14/36. The testis receded into the canal a short time after treatment was stopped.

Fig. 2. I.K., aged 11 yr. Showing well marked increase in size of genitalia during administration of 12,900 R.U. of follutein in 8 months. In spite of this growth, descent of the right testis failed to occur until operative procedures were carried out. *A).* 5/2/35. Before treatment. *B).* 11/15/35. During treatment. *C).* 2/14/36. After operation.

Fig. 3. C.DiB., aged 7 yr. Showing increase in size of genitalia during administration of 5,440 R.U. of follutein in 2 months. In spite of growth, descent of the testis did not occur without operation. *A).* 11/13/35. *B).* 2/14/36.

Fig. 4. L.O., aged 10 yr. Showing increase in the size of the genitalia during the administration of 6,350 R.U. of follutein, after an attempt, in another clinic, to bring a right undescended testis into the scrotum with operation. *A).* 11/12/35. Before treatment. *B).* 3/14/36. During treatment.

FINDINGS AT OPERATION

Seven of the patients in whom descent failed to occur were later operated upon and in each instance anatomical factors were found at the operation which prevented descent. A résumé of these findings is given below.

R.B., aged 8 yr. After receiving 8,000 R.U. of follutein in 3½ months, a right orchidopexy was performed November 16, 1935 by Dr. A. D. Bevan. The right testis was not palpable before operation. A long process of peritoneum was found

coming from the external ring. As this was followed to the internal ring, the testis was found just inside the abdominal cavity. By simply separating the peritoneum from the cord and severing it transversely, the cord was lengthened sufficiently to bring the testis into the upper part of the scrotum. Descent was prevented by the shortness of the peritoneal process and the 3 fascial layers attached to the cord.

M.A., aged 12 yr. Oct. 18, 1933, at the age of 10 yr., a right orchidopexy had been performed by Dr. A. D. Bevan. A small testis was found in the middle of the canal. The location of the peritoneal process was not described. Constrictive bands of fibrous tissue were found within the cord itself and, when severed, allowed sufficient lengthening for the testis to reach the scrotum easily. Treatment with follutein was started July 31, 1935. At this time the left testis could not be felt. After a total of 8,175 U. in 4¾ months, the left testis could sometimes be felt high in the inguinal canal when the patient stood up. A left orchidopexy was done by Dr. Bevan on Dec. 26, 1935. There was no obvious external ring, but only a few fibres of connective tissue in its usual position. On opening the external oblique the left testis and covering of peritoneum were found on the surface of the internal oblique above Poupart's ligament. With little effort, except freeing of fine connective tissue bands, the testis could easily be placed in the scrotum. The cord and testis seemed to be somewhat enlarged. Descent was prevented by the shortness of the peritoneal process and the 3 fascial layers adherent to the cord and testis, as well as by an absent or deficient external ring.

T.G., aged 8 yr. After receiving 4,590 U. of follutein in 4½ months, a left orchidopexy was performed April 6, 1936 by Dr. A. D. Bevan. Under the superficial fascia was found a large sac which covered the testis. The sac appeared to lie loosely under the fascia. It had passed through a normal sized external ring and lay above and lateral to the ring. In the process of dissection, a band of connective tissue was found at the lower end of the peritoneal pouch, which went down to the neck of the scrotum. It may have been gubernaculum testis. Because of the anatomic location with the sac turned upward on the external oblique, it would appear that the testis would have moved upward and not downward, with any growth. The reason for this condition is not clear, but it is a fairly common finding at operation. In this case descent was also prevented by the shortness of the structures to which the testis and cord were attached, as well as by the anatomical position just described.

I.K. (fig. 2), aged 11 yr. before treatment. The right testis was high in the inguinal canal, but could be displaced down to the upper part of the scrotum. Although the genitalia increased markedly in size during 8 months of treatment with follutein (12,900 R.U.), the position of the right testis did not vary. Right orchidopexy was performed Jan. 4, 1936 at the age of 12 years, by Dr. A. D. Bevan. The testis was found lying just under the external ring in the peritoneal pouch, which extended on downward until it ended at the neck of the scrotum as a fibrous cord. By stripping the peritoneum off the cord, and severing it, sufficient length was obtained to permit the testis to lie in the scrotum. The surgeon noted that there appeared to be more vascularity of the cord and surrounding structures than in patients not receiving APL substance. Descent was prevented by the shortness of the structures to which the testis and cord were attached.

C.DiB. (fig. 3), aged 7 yr. After 5,400 U. of follutein in 2 months, right orchidopexy was performed by Dr. Gatewood Oct. 21, 1935. Although the testis could be felt in the inguinal canal and pushed down to the upper end of the scrotum before treatment, it failed to descend until operative procedures were carried out.

The testis was found in the canal bound to the peritoneal pouch and three fascial layers by connective tissue. The reason for the failure of descent was the same as in the first case.

P.L., aged 11 yr. The testis showed no change after 7,740 U. of follutein in 5½ months. Left orchidopexy was performed by Dr. A. D. Bevan Jan. 8, 1936. The testis was found under the external oblique at the external ring. The peritoneal pouch extended to within 1½ cm. of the neck of the scrotum. It did not end in a fibrous cord, but lay up over the rectus sheath. There appeared to be three possible factors influencing descent: *1)* Shortness of structures to which the testis and cord were attached. *2)* Abnormal location of the peritoneal pouch. *3)* Apparent absence of fibres of the gubernaculum going into the scrotum.

J.D.P., aged 17 yr. The testis showed no change after 5,200 R.U. of antuitrin-S in 1 month. Right orchidopexy was performed by Dr. N. J. Heckel, June 18, 1936. The right testis and peritoneal process were turned upward on the external oblique. Descent was prevented by adhesions of the testis and cord to the fascial layers and by the abnormal location and direction of the peritoneal pouch and testis. The condition was virtually the same as that found in T.G.

Upon reviewing these results, it may be noted that in all cases descent was prevented by the shortness of the structures to which the cord and testis were attached, namely, the peritoneal process, the transversalis fascia, the cremasteric muscle and fascia and the intercolumnar fascia. In addition, the peritoneal process was directed abnormally in 3 cases and there was an absent external ring in 1 case. The growth of the genitalia in these patients (fig. 2, 3 and 4) together with the prompt descent of the testes in those in whom successful results were obtained, support the conclusion that the testes failed to descend for purely anatomical reasons. While the original cause of failure of descent in the majority of cases may have been a lack of adequate hormone stimulation, it is possible that as time progressed the structures involved in descent became less and less susceptible to the growth stimulus and the testes became fixed in their abnormal positions. Eccles (31) has pointed out that failure of descent may be caused by abnormality of any of the parts involved in descent, notably by conditions associated with the testis and its component parts; by conditions associated with the gubernaculum testis; and by conditions associated with the route of descent. He points out that the ring is formed around the gubernaculum and the gubernaculum in its early development is attached to the various structures recorded in the section on embryology. He further points out that the anatomical position of undescended testes conforms in the majority of instances to the lower attachment of the fibres of the gubernaculum. It is possible that failure of development of the fibres attached to the lower part of the scrotum or abnormal development of others may account for some cases of failure of descent. Various anatomical reasons for failure of descent are also reviewed by McCollum (32).

It is our impression that the problem will be thoroughly understood only after more careful attention is given to all the embryological and anatomical factors involved. Careful recording of operative findings in all cases in which descent fails to occur with treatment will be of value.

One of us (A. D. Bevan) desires to express separately the following opinions concerning the value of the anterior pituitary-like principle in the treatment of undescended testes. "The research is as yet in the experimental stage. There is evidence that this principle is a growth stimulant to the male genitals. The reports in the literature of its powers of producing rapid descent in a large percentage of cases appear to be exaggerated. Further experiments with this agent in a large series of cases by men who are thoroughly familiar with the anatomy, the embryology, the clinical history and course, and the operative treatment should be made in order to determine scientifically its value, if any. There is as yet an uncertainty as to the risks and the value of bringing on changes which simulate an artificial puberty in a child of 7 or 8 years. In our series I have not seen any evidence of injury and feel that we are warranted in continuing the use of this agent as an experiment. If its use is followed by descent, well and good. If after 6-months' trial no change occurs, the child should be given the benefit of an operation with the purpose of bringing the testis into the scrotum before the advent of puberty."

SUMMARY

The effects of treatment with the APL substance from the urine of pregnant women has been observed in 21 undescended testes in 18 boys from 1½ to 17 years of age. In 8 instances the testes were intra-abdominal, and in 13 inguinal. The dose of APL substance varied, but commonly was about 200 R.U. 3 times a week for an average of 5 months.

Descent occurred in 4 patients (19 per cent). In all 4 the testis was in the inguinal canal before treatment, and in 2 could be pushed to the upper end of the scrotum. Descent occurred within 1 month in all 4. In 1 of these 4 patients the testis returned to the inguinal canal when treatment was stopped.

In 7 other patients, treated surgically, after prolonged administration of APL substance, anatomical factors making descent difficult or impossible were present. However, preoperative treatment with this material caused definite stimulation of genital growth and thus seemed to make surgical procedures less difficult.

Operative procedures appear to be necessary in most cases of undescended testes, but in the present state of our knowledge, should be preceded by from 4 to 6 months of treatment with APL substance.

REFERENCES

1. Crowe, S. J., H. Cushing, and J. Homans: Bull. Johns Hopkins Hosp. **21:** 127, 1910.
2. Aschner, B.: Arch. f. Gynäk. **97:** 200. 1912.
3. Zondek, B.: Ztschr. f. Geburtsh. u. Gynäk. **90:** 372. 1926.
4. Smith, P. E.: Proc. Soc. Exper. Biol. & Med. **24:** 131. 1926.
5. Smith, P. E., and E. T. Engle: Am. J. Anat. **40:** 159. 1927.
6. Evans, H. M., and J. A. Long: Proc. Nat. Acad. Sc. **8:** 38. 1922.
7. Moore, C. R., G. F. Simmons, L. J. Wells, M. Zalesky, and W. O. Nelson. Anat. Rec. **60:** 279. 1934.
8. Aschheim, S., and B. Zondek: Klin. Wchnschr. **7:** 1404; 1453. 1928.
9. Schapiro, B.: Deutsche. med. Wchnschr. **56:** 1605. 1930.
10. Bevan, A. D.: J.A.M.A. **33:** 773. 1899.
11. Bevan, A. D.: Ann. Surg. **81:** 847. 1925.
12. Bevan, A. D.: Surg. Clinics of N.A. **10:** 193. 1930.
13. Huberman, J., and H. H. Israeloff.: J. Pediat. **7:** 759. 1935.
14. Aberle, S. B. D., and R. H. Jenkins: J.A.M.A. **103:** 314. 1934.
15. Brosius, W. L.: Endocrinology **19:** 69. 1935.

16. Cohn, S.: J.A.M.A. **103:** 103. 1934.
17. Dorff, G. B.: Am. J. Dis. Child. **50:** 1429. 1935.
18. Goldman, A., and A. Stern: New York State J. Med. **33:** 1095. 1933.
19. Kuhnstadter, R. H., and L. S. Robins: J. Pediat. **4:** 774. 1934.
20. Kuhnstadter, R. H.: Am. J. Dis. Child. **51:** 229. 1936.
21. Priestley, J. B., and R. Cohen: J. Iowa M. Soc. **25:** 547. 1935.
22. Rubinstein, H. S.: Endocrinology **18:** 475. 1934.
23. Sexton, D. L.: Endocrinology **18:** 47. 1934.
24. Sippe, C. A.: M. J. Australia **2:** 787. 1935.
25. Spence, A. W., and E. F. Scowen: Lancet **2:** 1335. 1935.
26. Webster, B.: J.A.M.A. **104:** 2157. 1935.
27. McKenna, C. M., and E. Ewert: J.A.M.A. **105:** 1172. 1935.
28. Brosius, W. L., and R. L. Schaffer: J.A.M.A. **101:** 1227. 1933.
29. Heckel, N. J.: Urol. & Cutan. Rev. **39:** 463. 1935.
30. Charny, C. W.: J. Urol. **32:** 217. 1934.
31. Eccles, W. M.: Brit. M. J. **1:** 503; 570. 1902.
32. McCollum, D. W.: Arch. Surg. **31:** 290. 1935.

EVALUATION OF HORMONE THERAPY FOR UNDESCENDED TESTES IN MAN

ARTHUR J. CRAMER, JR.
From the Children's Hospital
BUFFALO, NEW YORK

In 1902, Eccles (1) described the physiology of the imperfectly descended testis and suggested that the condition was linked with the glands of internal secretion. Not until recent years have commercial hormone products been available to test the correctness of his hypothesis. Much has been written about the treatment of cryptorchidism in the human but only in the past 3 years has endocrine therapy been suggested as another mode of treatment. Although the reported number of cases thus treated is comparatively small there is sufficient evidence of the efficacy of endocrine therapy to warrant its serious consideration. The purpose of this paper is to review briefly the subject of cryptorchidism and to summarize cases reported by other workers. Twenty cases treated in the Endocrine Clinic of the Children's Hospital of Buffalo during the past 2 years are added to the series.

Incidence. Cryptorchidism is more frequently encountered in children than in adults, a fact which would indicate that in a considerable number of cases descent of the testes takes place spontaneously about the time of puberty. However, among army recruits during the World War the condition was found to exist in 2 to 5 individuals per 1,000 (2). Of these, 25 per cent were bilateral maldescent.

Drake (3) has recently compiled a series of 11 cases from the records of some 260 physical examinations of school boys between 9 and 19 years of age. A second series of 268 boys between 9 and 20 years of age, revealed 16 cases of maldescent of the testes, 8 of which were bilateral.

Physiology. The exact mechanisms involved in the descent of the testes are not understood but Engle (4) suggests it is probably the result of 3 primary factors. First, one must consider the gubernaculum testis. This ligament extends from the caudal pole of the testis through the inguinal canal to the inferior scrotal wall. During the 7th lunar month of gestation, the gubernaculum not only ceases growth, but actually shortens one-half of the length. The resultant shortening, both actual and relative, serves to draw the testis into the scrotum where it is usually found at the 9th lunar month. Once descended, the testicles are usually prevented from returning into the abdominal cavity by the contractive action of the muscles surrounding the inguinal canal.

The second factor is the mechanical effect which may be explained on an anatomical basis. The inguinal canal is formed by reflection of muscles and fascia and since Engle (4) has shown that man and apes are the only animals that have the internal ring closed at birth, one may consider the shape of the canal as that of an inverted funnel. Hence, as the testis grows it is forced into the scrotum.

The gonadotrophic hormone liberated by the anterior pituitary may be the third

factor. Arey (5) in his work on the causes of testicular descent, makes no note of hormonal influence. It is evident from the lack of specialized tissue in the gubernaculum testis that the structure would not be responsive to hormonal stimulation. On the other hand, it has been conclusively demonstrated (6, 7, 8) that a marked increase in growth of the interstitial tissue of the testicle occurs following the injections of hormone recovered from the urine of pregnancy.

Probably the best conception of testicular descent is that it is the result of the 3 factors: the hypertrophy of the testes under hormone stimulation; a mechanical directive action produced by the funnel-shaped enclosure; and a possible tractor influence of the gubernaculum testis.

Pathology. At birth both testes are found normally in the scrotum. Incomplete descent of the testes may exist in a varying degree from an abdominal position to one at the external ring. Moore (9), Wangensteen (10) and Cooper (11) in separate investigations have greatly clarified the effect of position on pathological changes produced in the testes. Moore and others have established the fact that the abdominally placed testis undergoes marked degenerative changes. Wangensteen, working with dogs, confirmed the work of Moore and also found the degenerative changes occurred only after puberty had been reached. Cooper studied testes of cryptorchid children and concluded that some anatomical defect was almost always found after 3 years of age. There appears to be justification for the conclusion of Aberle and Jenkins (12) that the sooner the undescended testis is placed in the scrotum the better are the chances of its being a normal organ.

Unilateral maldescent is usually due to mechanical obstruction. Congenital malformation or fibrous bands may be the cause of the obstruction. Eisendrath (13) believes the internal oblique muscle of the normal person acts as a sphincter to prevent retraction of the testes by the cremasteric muscle. At operations for undescended testes he has frequently found faulty development of the arching fibres of the internal oblique and a weak conjoined tendon which he believes plays an important role.

Bilateral cryptorchidism may be a primary condition or it may be secondary to a generalized endocrine dysfunction. In the present series of cases about fifty per cent had undescended testes as a feature of an obvious hypopituitary condition.

EXPERIMENTAL WORK IN ANIMALS

Engle (14), in 1929, noted that there was a slight but variable acceleration of the growth of the sex apparatus when extracts of the urine of pregnancy were injected into immature rats. DeJongh (15) noted that the hormone of pregnancy urine caused an increase in the growth of the secondary sex characteristics of mice and young rats provided normal or unilaterally castrated animals were used; the injections had no effect on total castrates. From examination of the testes he concluded the action was on the testes, that it seemed to be confined to an interstitial tissue stimulation of that organ and that no change in spermatogenesis was observed, even after large doses. Bourg (16) further confirmed the observation of deJongh. Because irradiation of the testes produces destruction of the germinal epithelium and leaves the interstitial tissue intact, he injected irradiated rats with extract of pregnancy urine. The same increased growth of the secondary sex characteristics in these animals as in normal or uni-castrates was produced. He concluded, therefore, that the active substance of the urine of pregnancy produces its effect directly upon the interstitial cell mass of the testes with a resultant development of the genital tract and of the accessory sex apparatus. Molien (16) believes that the function of this hormone,

since spermatogenesis is not hastened, is only to increase the output of the so-called male sex hormone of the testes and thus secondarily causes the full development of the sex apparatus.

In contrast to the work of Engle, deJongh, and Bourg who found that spermatogenesis is not effected by the extract of urine of pregnant women, Schockaert (17), Balawenetz (18), Kraus (19) and Butcher (20) working independently with a variety of species of animals have reported opposite results. Brosius and Schaffer (21) in this country and Schapiro (22) abroad have reported a few convincing results in humans and as the former has stated, man may be an exception to the general rule.

In spite of these conflicting statements it is generally accepted by these workers that the hormone of the urine of pregnancy exerts its primary effect on the interstitial tissue of the testes and hence governs the development of the accessory sex apparatus.

As early as 1932 Engle (4) concluded that, since the normal size of the testicle is due to this hormone action and since such stimulation is at least the exciting factor in the normal descent of the testes, operative procedures for the placement of an undescended testis in the scrotum are of questionable value.

Smith and Engle (23) in 1927 compared the results of direct anterior pituitary substance transplants into the muscle of the recipient rat with the influence exerted by the gonadotrophic hormone of the urine of pregnancy injected intramuscularly. Although there was a parallelism of effects, the animals reacted to the urinary extract to a greater degree than to fresh pituitary transplant. Despite the similarity of the response, it was not considered by these workers, nor later by Moore and Price (7), as evidence that the urinary principle is of hypophyseal origin.

CLINICAL RESULTS IN MAN

Since Schapiro's (22) first report in 1930 several investigators have reported small series of cases treated by hormone therapy. Because of the relatively low incidence of cryptorchidism it has been difficult for any one man to present a large group of cases. It seems worth while, therefore, to summarize the cases published to date, adding 20 more, making a total of 81 cases. This series is sufficiently large to be accepted by the most critical.

Table 1 shows the essential facts of published case reports up to Nov. 1935. Dosage in R.U. is used in an attempt to standardize the amount of treatment necessary to bring results. Frequency of administration and age for treatment have been variable among the various workers and a summary of such may help one arrive at proper conclusions. Results are divided into 3 columns, namely, complete, partial and failure. By complete result is meant that both testes came to rest in the lower scrotum; partial, that the testes moved from their original position but one or both still were not fully descended; the failures are those cases in which no change was noted.

Schapiro (22) published the first article concerning the effect of the gonadotrophic hormone of urine of pregnancy on 80 cases of hypogenitalism. He wrote with much enthusiasm because of the striking results obtained. Much of the publication was devoted to the general endocrine picture and no detailed reports were given in 13 individuals of his total group. Prähormone made from the urine of pregnant women was the product employed and was standardized to contain 100 R.U. per cc. In general his total dosage and frequency of administration were greater than those used by other workers.

Dorff (30) in a recent publication presented results similar to those obtained by Shapiro (22). In 6 of Dorff's 14 patients no maldescent of the testes was present but a marked hypogenitalism did exist. Striking results in these subjects were obtained, as evidenced by a 3-fold increase in the size of the testes, penis and scrotum and a growth of pubic hair. The remaining 8 cryptorchid cases of this investigator are included in the summarized chart.

Since the table is self-explanatory only general statements need be made concerning the reports of other workers. In this country at least, the hormone extract employed was either antuitrin-S (Parke, Davis & Co.) or follutein (E. R. Squibb & Co.). Both commercial products are made in essentially the same manner from the urine of pregnant women and are standardized in R.U. Antuitrin-S contains 100 R.U. per cc. and follutein 250 R.U. per cc. The total dosage varied in the individual case but 1,000 to 3,000 R.U. was sufficient to bring results in the majority of cases. How large a dose is sufficient is difficult to state, apparently Brosius (28) used 75,000 R.U. without evident harm. The low percentage of failures as reported by some of the workers may be explained by the fact that their total dosage was greater than others. The frequency of administration was usually 2 to 3 times a week although daily injections were given in some cases. The age at which treatment was given varied from 3 to 38 years but the majority of patients were at pre-puberal ages. It is interesting to note that in over one-half of the total cases a general endocrine deficiency existed. Unilateral undescent of the testis appeared to be more common than the bilateral condition. Some attempt was made to classify the degree of cryptorchidism. In the entire series in which the position of 95 testes were described, over 50 per cent were abdominal in position before treatment was started. This fact should aid in eliminating the possibility of spontaneous descent. Evaluation of the end results of the various investigators is not without difficulty. To be absolutely fair in any given case, the result was not considered complete unless both testes after treatment were found in the lower scrotum. When bilateral undescended testes had existed and after treatment descent was obtained on one side only, the result was considered partial. Individuals in whom incomplete descent occurred following treatment are also included as a partial result.

The primary result of any therapy for cryptorchidism is the scrotal placement of the undescended testis. This object was attained in 58 cases or 71.6 per cent of the total 81 cases and a partial improvement in 16 per cent more cases, making a total of 87.6 per cent that were benefited by this form of treatment. Not only is this method of therapy conducive to scrotal position but also presents the additional advantage of correcting hypogenitalism when it is present.

PRESENT CASES

The following are the observations on 20 consecutive cases of undescended testes treated by hormone therapy since May, 1933. All except 3 cases,[1] were treated in the Endocrine Clinic of the Children's Hospital of Buffalo.

Before any treatment was given every effort was made to check the diagnosis

[1] Three cases by courtesy of Dr. William J. Orr.

TABLE 1. SUMMARY OF REPORTED CASES.

Author	Dose, Extract	Frequency	Age range years	Total No. of cases	Appearance		Occurrence		Degree of Undes.		Result		
					Normal	Endocrine type	Unilat.	Bilat.	Complete (Abdomen)	Partial (Canal)	Complete	Partial	Failure
Schapiro (22)	7–10,000 R.U. *Prähormone*. 1 cc = 100 R.U.	200–300 R.U. 3× weekly. Also daily.	9–27	13	2	11	—[2]	—[2]	—[2]	—[2]	10	3	0
Goldman-Stern (24)	Total dose not stated. *Antuitrin-S*. 1 cc = 100 R.U.	100 R.U. 3× weekly. Thy. and pit. ext. by mouth.	12–15	2	1	1	2	0	2	0	2	0	0
Sexton (25)	3–42,000 R.U. *Antuitrin-S*. and *Follutein*.	100–300 R.U. 2–3× weekly. Also daily.	10–18	6	1	5	3	3	5	4	4	1	1
Cohn (26)	1–2,600 R.U. *Antuitrin-S*.	100 R.U. 3× weekly.	7–12	6	4	2	4	2	4	4	5	1	0
Rubinstein (27)	24,600 R.U. *Follutein*. 1 cc = 250 R.U.	50–250 R.U. daily Pit. and thy. ext. by mouth.	10½	1	0	1	0	1	2	0	0	1	0
Aberle-Jenkins (12)	1,700–4,500 R.U. Product not stated.	50–200 R.U. 3× weekly.	3–13	4	1	3	3	1	1	4	2	0	2
Brosius (28)	500–6,000 R.U. 1 Case. 75,000 R.U. *Antuitrin-S*.	100–300 R.U. 2–3× weekly.	3–13 1 case 38	10	3[1]	3[1]	6	4	4	8	6	2	2
Webster (29)	1–6,000 R.U. *Follutein*.	25–250 R.U. daily.	6–26	11	6	5	8	3	7	7	9	1	1
Dorff (30)	8–45,000 R.U. Product not stated. 1cc = 250 R.U.	125–375 R.U. 3× weekly. Also daily, 7–10 months.	6–12	8	0	8	4	4	3	9	7	0	1
Cramer	500–5,000 R.U. *Antuitrin-S*.	100 R.U. 2× weekly.	5–13	20	14	6	10	10	24	7	13	4	3
		Total		81	32[1]	45[1]	40[2]	28[2]	52[2]	43[2]	58 71.6%	13 16.0%	10 12.4%

[1] Four cases not stated.
[2] Thirteen cases not stated.

and record the accurate location of the undescended testis. At least 2 different examiners on 2 different occasions carefully examined the patient. If the position of the testis could not be determined upon inspection, the tip of the examiner's little finger was placed into the external inguinal ring as far as possible and the testis forced down from above with the other hand. In recent months it has been the practice in the out-patient department to first refer all cases of undescended testes to the surgical clinic. This has offered a further check on the pre-treatment examination.

Only collective statements and special observations are described in table 2 to avoid repetition of case detail. The majority of the cases treated were from 9 to 13 years of age. Previous to the advent of hormone therapy this age period had been the desirable time by most surgeons for the surgical correction of the undescended testes. Cryptorchid children under 9 years of age until recent months were given no treatment but were advised to return at a later date. Since results obtained by hormone therapy have been so encouraging and no apparent harm has been observed, younger children have been started on treatment. Antuitrin-S (Parke, Davis & Co.) has been the urinary extract employed in the entire series. A few cases also received thyroid extract by mouth and whole anterior pituitary extract by injection but the effect of these extracts on the results obtained is not known. The total dose required to produce results varied from 500 to 5,000 R.U. Injections of 100 R.U. (1 cc.) were given twice weekly in most cases. When this method of treatment was first employed it was given in courses of 10 injections (total 1,000 R.U.), that is, twice weekly for 5 weeks, with 2 to 3 monthly rest periods between courses. No particular advantage was observed in monthly rest periods and treatment is now given continuously. Under the classification of general appearance, children that are described as normal are those in which the physical examinations including height and weight, according to Englebach's figures, are within normal limits. Hypopituitary is the name used to describe the picture of obesity associated with hypogenitalism. Mild or masked hypothyroid children which were diagnosed as such, by evidence of retarded epiphyseal centers and were subnormal in height, are described as 'small size.'

Rapidity of response to this mode of treatment for undescended testes has shown a marked variation. Approximately one half of the cases have shown results after 500 to 1,000 R.U. were injected. The remaining one half were much slower in response or no change at all was observed. Probably the most dramatic result was seen in the first case (A.R.). He was given large doses of whole anterior pituitary beef extract. This product was prepared locally and by animal experimentation had proved to be a very potent extract. Before any treatment was started on this patient neither testis could be found after several examinations. Because of the supposed potency of the newer extract, 60 injections were given over a period of 7 months. He was re-examined at least 30 times during this treatment and no change was observed. All treatment was stopped for 2 months and then antuitrin-S, 100 R.U. twice weekly, was administered. After the third injection the left testis was found in the scrotum and the right testis in the canal. However, it required 3,700 R.U. (37 injections)

TABLE 2. CASES TREATED AT THE CHILDREN'S HOSPITAL OF BUFFALO, N. Y.

Case and Age	General Appearance	Position of Undes. Testes	Total Dose R.U.	Frequency	Result	Remarks
(1) A.R. 10 yr	Normal	Both in abdomen	4,000	100 R.U. 2×wk. for 5 wks. with 3-2 mo. rest periods	After 300 R.U. L.T. in scrotum and R.T. in canal. After 4000 both in scrotum.	Previously had rec'd 60 inj. of whole beef ant. pit. ext. with no result. Pain in groin.
(2) J.E. 9 yr.	Normal	Both in abdomen	1,100	100 R.U. 2×weekly for 5 wks.	After 700 R.U. L.T. in scrotum R.T. in canal. After 1, 100 both in scrotum.	Checked 6 mo. later; both testes in scrotum.
(3) J.V. 12 yr.	Normal	R.T. in canal	1,000	Same	After 1,000 R.U. R.T. in upper scrotum.	Left testis larger; 4 mo. later both in scrotum. Pain in groin.
(4) J.D. 9 yr.	Hypopitultary	Both in abdomen	1,000	Same	After 500 R.U. L.T. in scrotum. After 800, R.T. in scrotum.	Weight loss 6 lb. Genitals increased.
(5) R.G. 12 yr.	Hypopitl.itary	R.T. in abdomen; L.T. in scrotum, very small.	2,000	100 R.U. 2×weekly for 10 weeks.	After 1,700 R.U. R.T. in scrotum. L.T. 2× size.	Weight loss 18 lb. Genitalia increased.
(6) R.C. 9 yr.	Normal	Both in abdomen	1,000	100 R.U. 2×weekly for 5 weeks.	After 400 R.U. R.T. in scrotum. After 600, L.T. in scrotum.	Stopped bed-wetting
(7) B.A. 7.5 yr.	Mild Hypopitultary	Both in abdomen	1,000	Same	After 500 R.U. both at ext. ring. After 800 both in scrotum.	Weight loss 3 lb. Some pain in groin.
(8) B.P. 11 yr.	Hypopitultary	Both in abdomen	3,800	100 R.U. 2× weekly for 5 wks. with with monthly rest period.	After 900 R.U. L.T. in scrotum. After 2,900, R.T. in scrotum.	Weight loss 10 lb. Increase in penis and testicles. Fat redistribution.
(9) W.E. 9.5 yr.	Normal	R.T. in abdomen	500	100 R.U. 2× weekly for 2½ weeks.	After 300 R.T. in canal. After 500, in scrotum.	Pain in groin.
(10) A.S. 10.5 yr.	Normal	R.T. in lower canal	1,000	100 RU. 2× weekly for 5 wks.	After 800 R.U. R.T. in scrotum.	Hernia on left but testes in scrotum.
(11) C.C. 12 yr.	Normal	R.T. in abdomen	600	100 R.U. 2× weekly.	After 500 R.U. in scrotum.	
(12) C.M. 12 yr.	Normal	L.T. in abdomen; R.T. low in canal assoc. with hernia.	1,000	100 R.U. irregularly for 2 mo.	After 1,000 R.U. in scrotum; L.T. no change.	Poor cooperation. Large hernia on right.
(13) C.T. 13 yr.	Normal	L.T. in canal assoc. with hernia.	400	100 R.U. irregularly for 3 weeks.	No change	Poor cooperation.
(14) N.C. 12 yr.	Normal	R.T. in abdomen	1,000	100 R.U. 2× weekly for 5 wks.	R.T. larger in lower canal after 1,000 R.U.	On rest period penis enlarged; pubic hair appearing; had pain in groin.
(15) L.V. 10 yr.	Small size	Both in abdomen	5,000	100 R.U. 2× weekly for 5 wks. 1 mo. rest periods.	After 2,100 R.U. R.T. at int. ring. After 5,000 in lower canal.	Hypertrophy of penis. Now on rest period.
(16) W.N. 9 yr.	Normal	R.T. high in canal.	1,000	100 R.U. for 5 wks. 2× weekly.	No change.	Now under tr.
(17) L.Z. 5 yr.	Normal	L.T. in abdomen; R.T. high in canal.	1,000	100 R.U. 2× weekly for 5 wks.	After 500 R.U. both in lower canal. After 1,000 both in scrotum	Still under tr.
(18) C.L. 12 yr.	Early Hypopitultary	Both in abdomen; R.T. operated.	5,000	100 R.U. 2× weekly. Thy. ext. ant. pituitary also.	R.T. in upper scrotum but very small.	R.T. enlarged 3×. Penis and scrotum increased. No effect on left. Wt. loss 20 lb.
(19) M.C. 5 yr.	Normal	R.T. in canal after operation.	1,000	100 R.U. 2× weekly for 5 wks.	R.T. in scrotum af- 500 R.U.	Testes slipped back in canal before antuitrin started.
(20) S.L. 11 yr.	Small size	R.T. in abdomen.	2,000	100 R.U. 2× wkly. Thy. ext. ant. pituitary.	No change.	

before the right testis rested in the scrotum. It is difficult to believe this case was the result of spontaneous descent.

Results obtained in case C.L. were also interesting. About 6 months previous to the advent of hormone therapy for undescended testes, he was subjected to a surgical correction on the right side. Cryptorchidism had existed bilaterally and the penis and scrotum were undersized. At operation the right testis was found high in the canal and was somewhat smaller than normal. During the 6 months following operation the right testis atrophied slightly and remained in the upper one-third of the scrotum. Antuitrin-S was then given with the hope of bringing down the left testis, but no evidence of it could be found after 5,000 R.U. However, the right testis tripled in size, the penis and scrotum enlarged, pubic hair began to appear and fat redistribution with some weight loss resulted. These changes were significant and the case was considered a partial result.

Case M.C. was another in which hormone therapy was given following surgical correction. A large hernia with an undescended testis originally existed on the right side. After operation the testis retracted to a position high in the canal. Hormone treatment brought the testis back to a scrotal position where it has remained to date.

Case A.S. had a hernia on the left side and an undescended testis on the right. Although surgery was expected for the repair of the hernia, antuitrin-S was employed pre-operatively for the cryptorchid testis. A prompt satisfactory result was obtained for the testicular condition and bilateral surgery was not necessary.

Nearly all the cases in which a result was obtained showed an increase in the size of the testis, penis and scrotum. Very often the testis which descended was at first quite small and when examined at a later date was found to have doubled the original size. Scrotal pouches which were originally very shallow with 2 or 3 folds, were increased in depth and in the number of folds. Unilateral cryptorchid patients often showed an increase in the size of the testis on the normal side. Six patients reported occasionally a slight pain in the groin during the 24 hours following an injection.

Body weight was unaffected in the normal individuals. However, in about one-half of the hypopituitary children, some weight loss occurred. The fat redistribution in these individuals was even more striking than the actual total weight loss in pounds.

Most of the patients in whom a partial result or a failure of treatment were reported, are still under therapy. Since other investigators have employed a higher total dosage to produce results, it is possible that treatment in the future may still give satisfactory response in these cases. The entire series is still under observation and in no patient has cryptorchidism recurred after hormone therapy had produced satisfactory results.

COMMENT

Until recent years, the physician had the choice of two methods of therapy in the treatment of cryptorchidism. He either waited for spontaneous descent of

the testes or subjected the patient to a surgical correction. Hormone therapy now offers a third possibility but has not as yet gained universal acceptance. A fourth method which employs mechanical suction has very recently been described by Ballenger, Elder and McDonald (31).

Spontaneous descent of the testes from birth to puberty does occur and deserves serious consideration. Many of us have seen individual cases in which this has taken place. Drake (3) reported 35 cases of undescended testes in boys, 23 of which showed a spontaneous descent mostly at the time of puberty. He believes that uncomplicated undescended testes do not present any problem, especially of a surgical nature. The only cases that he would consider as suitable for surgical repair are the bilateral type that persist after the 14th year or the unilateral undescended testis that presents some complication, such as torsion of the cord, hydrocele, malignancy, etc.

The investigations of Moore (9), Wangensteen (10) and Cooper (11) on the histology of the retained testes has been mentioned. Their observations make one question the advisability of waiting for spontaneous descent of the testes. The work of Wangensteen confirmed the opinion that it was safe to use expectant treatment up to the time of puberty. On the other hand, Cooper observed abnormal changes in the undescended testes after 3 years of age. These changes observed by the latter were much less striking in the testes of young boys than in those who had passed through puberty. Therefore, it may be concluded that treatment is not necessary in young children but should be carried out before the onset of puberty.

In considering the surgical aspect, one finds that writers on the subject of orchidopexy for correction of cryptorchidism are not in agreement as to the ideal age for operation. Bevan (32) advises that the operation be performed the first or second year of life, for he doubts that the testis ever descends spontaneously after that time, unless it is already palpable and can be forced manually into the scrotum. Cabot (33) is of the opinion that the operation should be performed before the age of 9 years, and that the earlier it is done, the better the results will be. Also in favor of a very early age are Welti (34) and Higgins, while Wangensteen (10) believes that the age of 8 to 11 is the ideal time, especially in cases where the testes are not palpable either in the canal or in the scrotum.

Surgery may give a good anatomical result in the correction of an undescended testis but does not always insure a normal functioning organ. In a follow-up study of 15 operations for undescended testes Goetsch (2) reported that the testicle developed normally in only 1 instance. In a more extensive survey of some 537 orchidopexies, Burdick and Coley (35) were of the opinion that 50 per cent of the operations proved satisfactory as to position, and that in about 15 per cent of these the testes were of normal size. Cabot took exception to their opinion and offered the results of 2 analyses of cases from the Mayo Clinic, one by Counseller (36) and one by Walters and Thiessen (37). The analyses showed that good results were obtained in 92 to 93 per cent of the cases. It is the opinion of Cabot (33) that 4 of 5 cases will have satisfactory results by operation.

The third type of treatment, that of hormone therapy, is now more than

just an experimental method. The question arises, what harm may come from this form of therapy? This cannot be answered completely at the present time. However, in my experience, certain theoretical objections to this method of treatment are not met in practical application. Lower (38) and Johnson have shown that the maintenance of the normal prostate gland is dependent upon the secretion by the testes of the so-called 'male sex hormone.' Geschickter (39) and his co-workers, using the extract of the urine of pregnancy on young male monkeys, were able to produce hypertrophy of the prostate and enlargement of the breasts simulating a condition of gynecomastia. Hypertrophy of the prostate in 1 monkey was of such a degree as to cause urinary retention and dilatation of the bladder. It is true that in humans also, the hormone in addition to its effect in correcting the cryptorchid state, causes an increase in growth of the sex apparatus and secondary sex characteristics. However, in the reported cases and in my own series there has been no evidence of overstimulation of the accessory sex apparatus nor of breast activity. There is, no doubt, the possibility of over-stimulation if correspondingly large doses were used in man. Since these objections based on animal experimentation are not found in human application, hormone treatment should not be restricted in its use as a mode of therapy for cryptorchidism.

The use of mechanical suction apparatus for hypogenitalism and crytorchidism offers a fourth method of therapy. Although Ballenger (31) et al. have used this method for 15 years, the results have been just recently published. In their hands the results have been very satisfactory in both underdeveloped genitals and undescended testes. Descent of the testes was obtained in 23 of 26 cases. The method has its limitations, since no effect was observed in cryptorchid patients unless the testes were in the canal at the onset of treatment. Another disadvantage was that treatment was ineffective at or after the time of puberty. Since other workers have not reported on the use of this method and the series already published is small, no definite conclusion can be made at this time.

In considering these 4 possibilities as methods of treatment for human cryptorchidism, an attempt should be made to choose a conservative method of therapy. Probably a combination of the first 3 methods is the best. The advent of puberty in boys is usually placed between 13 and 15 years of age. With the onset of puberty, at least, degenerative changes begin to take place in the retained testes. Therefore, it seems logical to choose 9 to 12 years of age as the pre-puberty period in which some effort should be made to correct the cryptorchid condition. Since the end results of hormone therapy appear to be better than those of surgical correction, the former should be used first. If hormone treatment fails to bring a satisfactory result then, there is still ample time in this pre-puberty period to perform an orchidopexy. When cryptorchidism is associated with hypogenitalism, surgery alone may not benefit the latter condition and the use of hormone treatment postoperatively should be employed.

The physician is rarely justified to assume a passive attitude and allow an individual to go through puberty without correcting the cryptorchid condition.

The likelihood of sterility presents the major argument for employing corrective measures. It is an accepted fact that adults having bilateral undescended testes are sterile. There are reported, however, rare instances in which cryptorchid testes produced normal spermatozoa. Torsion of the cord, trauma and malignant degeneration are known to occur more frequently in undescended testes than in normally placed organs. Psychic influence on the patient is sometimes mentioned as another argument for instituting corrective treatment.

SUMMARY

An attempt has been made to evaluate the use of hormone therapy for the correction of human cryptorchidism. A summary of the published case reports to date, including 20 cases presented herewith, makes a total of 81 cases in the literature. Analysis of the results show that a complete descent of the testes was obtained in 71.6 and a partial result in 16, making a total of 87.6 per cent that were benefited by this method of therapy. The evidence is sufficient to consider hormone treatment a valuable method of therapy for undescended testes. When surgical correction is indicated, the results may be enhanced by the use of hormone therapy as an adjunct.

REFERENCES

1. Eccles, W. M.: Lancet **1:** 569. 1902.
2. Goetsch, A.: Am. J. Surg. **12:** 63. 1931.
3. Drake, C. B.: J.A.M.A. **102:** 759. 1934.
4. Engle, E. T.: Endocrinology **16:** 506; 514. 1932.
5. Arey, L. B.: Developmental Anatomy. W. B. Saunders Co., Philadelphia, 1934.
6. Bourg, R.: Compt. rend. Soc. de biol. **104:** 109; 1046. 1930.
7. Moore, C. R., and D. Price: Am. J. Physiol. **99:** 197. 1931.
8. Riddle, O., and I. Polhemus: Am. J. Physiol. **98:** 121. 1931.
9. Moore, C. R.: Anat. Rec. **24:** 383. 1923.
10. Wangensteen, O. H.: Arch. Surg. **14:** 663. 1927. Surg., Gynec. & Obst. **54:** 219. 1932.
11. Cooper, E. R. A.: J. Anat. **64:** 5. 1929.
12. Aberle, S. B., and R. H. Jenkins: J.A.M.A. **103:** 314. 1934.
13. Eisendrath, D. N.: Ann. Surg. **64:** 324. 1916.
14. Engle, E. T.: Anat. Rec. **43:** 87. 1929.
15. deJongh, S. E.: Arch. néerl. de physiol. **15:** 470. 1930.
16. Molien, M., F. D'Amour, and R. G. Gustavson: Endocrinology **17:** 295. 1933.
17. Schockaert, J. A.: Anat. Rec. **50:** 381. 1931.
18. Balawanetz, S.: Virchows Arch. f. path. Anat. **278:** 383. 1930.
19. Kraus, E. J.: Klin. Wchnschr. **9:** 1493. 1930.
20. Butcher, E. O.: Anat. Rec. (suppl.) **54:** 49. 1932.
21. Brosius, W. L., and R. L. Schaffer: J.A.M.A. **101:** 1227. 1933.
22. Schapiro, B.: Ztschr. f. klin. med. **114:** 610. 1930.
23. Smith, P. E., and Engle, E. T.: Am. J. Anat. **40:** 159. 1927.
24. Goldman, A., and A. Stern: New York State J. Med. **33:** 1095. 1933.
25. Sexton, D. L.: Endocrinology **18:** 47. 1934.
26. Cohn, S.: J.A.M.A. **103:** 103. 1934.
27. Rubinstein, H. S.: Endocrinology **18:** 475. 1934.
28. Brosius, W. L.: Endocrinology **19:** 210. 1935.
29. Webster, B.: J.A.M.A. **104:** 2157. 1935.
30. Dorff, G. B.: Am. J. Dis. Child. **50:** 649. 1935.
31. Ballenger, E. G., Elder, O. F., and H. P. McDonald: South. Surgeon **4:** 297. 1935.
32. Bevan, A. D.: Ann. Surg. **90:** 847. 1929.
33. Cabot, H., and R. M. Nesbit: Arch. Surg. **22:** 850. 1931.
    Cabot, H.: South. Surgeon **4:** 331. 1935.
34. Higgins, C. C., and H. Welti: Surg., Gynec. & Obst. **48:** 536. 1932.
35. Burdick, C. G., and B. L. Coley: Ann. Surg. **84:** 867. 1926.
36. Counseller, V. S.: J. Urol. **30:** 327. 1933.
37. Walters, W., and N. Thiessen: Proc. Staff Meet., Mayo Clin. **10:** 9. 1935.
38. Lower, W. E., and R. L. Johnson: J. Urol. **26:** 599. 1931.
39. Geschickter, C. F., D. Lewis, and C. G. Hartman: Am. J. Cancer **21:** 828. 1934.

THE TYPE AND DEGREE OF GONADAL STIMULATION INDUCED IN HYPOPHYSECTOMIZED MALE RATS PARABIOTICALLY JOINED WITH CASTRATED, CRYPTORCHID, AND NORMAL PARTNERS

EUGENE CUTULY, D. ROY McCULLAGH AND ELIZABETH CUTULY
From the Department of Biochemical Research, Cleveland Clinic
CLEVELAND, OHIO

The results of various studies lead one to conclude directly or by inference that the anterior lobe of the hypophysis of castrated animals produces predominantly a follicle-stimulating principle (1, 2, 3, 4, 5, 6). On the other hand the results of the investigation of Evans et al. (7) and of Lipschütz and Villagran (8) suggest that the luteinizing ability of the anterior lobe of the hypophysis of rats increases following castration.

As this report is concerned with experiments on parabiotic rats, special attention is directed to other studies of a similar nature. Witschi and Levine (5) joined castrated male rats with hypophysectomized females. Shortly after castration of the male, vaginal smears from the hypophysectomized female showed only cornified cells; this condition, with a few interruptions, persisted for long periods of time. The ovaries of such females revealed only follicular growth or atresia, without luteinization. Essentially the same results were obtained in hypophysectomized female rats which had been parabiotically united with spayed females (9, 10). Thus, regardless of the sex of the castrated partner used in these studies, the ovaries of the hypophysectomized female partner showed only, or chiefly, follicular stimulation.

It was thought advisable to determine what effects were produced in hypophysectomized male rats parabiotically joined with castrated, cryptorchid, or normal male parabionts.

MATERIALS AND METHODS

Adult albino male rats were used in this study. Some were littermates, but most of them were selected from uniform stocks. Parabiotic unions were of the celio-anastomotic type. The animals were anesthetized with ether and one side of each was thoroughly washed with 70 per cent alcohol. The hair was not removed. A long incision in the skin was made between the neck and the thigh on each animal. A much shorter incision (about ½ inch) was made through the abdominal musculature and peritoneum. Interrupted sutures were used to join the muscle layers and peritoneum of each rat in such a way that a lumen remained between the peritoneal cavities. Wound clips were used to bring together the edges of the skin.

Included in this report are data obtained only from pairs, the members of which were healthy and vigorous. Data on pairs in which infections developed at the sites of operation or which died suddenly are not given. These pairs were of value, however, since the results obtained with them conformed essentially to those which will be presented.

Three types of parabiotic pairs were studied: *1)* castrated male + hypophysectomized male; *2)* cryptorchid male + hypophysectomized male; *3)* normal male + hypophysectomized male. The operations to which members of a pair were subjected were performed at different times; hypophysectomy, for instance, sometimes preceded parabiosis and sometimes was performed subsequently to parabiosis. The times of operation can be determined readily from table 1 by subtraction. For example, pair 91h-91c was in parabiosis for 48 days. As 91h was hypophysectomized for 41 days, it follows that this animal was hypophysectomized 7 days following parabiosis. Its partner, 91c, was castrated for 20 days. This animal, therefore, was castrated 28 days after parabiosis and 21 days after its partner had been hypophysectomized.

Hypophysectomies were performed by the method of Thompson (11). Removal of the pituitary was not considered complete in any instance unless: *1)* the parts aspirated into the suction tube at the time of operation could be pieced together to form macroscopically the entire gland; *2)* the autopsy weights of the adrenals and thyroids, which were found to be unaffected by parabiosis, were significantly decreased; and *3)* microscopic examination of serial sections of the sella turcica revealed no fragments of the pituitary gland.

At the end of varying periods the animals were killed with chloroform and their organs were weighed in the fresh condition. In view of the fact that various parts of the genital tract may respond quantitatively differently to the same hormonal stimulation, it seemed desirable to weigh the organs separately. For this reason the ventral and dorsal portions of the prostate (anterior and dorso-lateral portions, according to Walker, 12) were weighed separately, as were the seminal vesicles. Coagulating glands were not weighed.

The hypophyses and sellae turcicae were fixed in Zenker-formol, cut at 5 micra, and stained with Mallory's triple stain. This stain when properly employed has been found to give nice differentiation of the various cell types in the anterior hypophysis. All other tissues were fixed in Bouin's fluid, cut at 5 micra, and stained with ordinary hematoxylin-eosin or with iron hematoxylin and light green.

RESULTS

In table 1 are given the weights of the adrenals and genital organs of the various animals. As the weights of the genital organs in many instances were not a true index of the type and degree of hormonal stimulation, the histological appearance of the organs has been indicated. An adequate number of non-parabiotic castrated, cryptorchid, and normal control rats was available; but, as the organs of these non-parabiotic control rats were in every respect similar to those of corresponding parabiotic animals with two exceptions which will be mentioned, the data on control animals have not been included. As the effects of castration and cryptorchidism in rats are well known, and as these effects were not significantly modified by parabiosis, they will not be discussed. Data on non-parabiotic hypophysectomized controls also have been omitted because, in a number of cases, the hypophysectomized partner of a parabiotic pair failed to show signs of hypophyseal stimulation, and such non-stimulated pituitaryless animals can therefore be regarded as hypophysectomized controls.

In order that the data presented may be more readily evaluated, the following points should be emphasized. Hill's (13) studies on parabiotic rats indicated that hormones pass from one partner to the other through anastomosing blood capillaries. He found no difference in response in test parabionts

TABLE 1. AUTOPSY DATA.

Pair	No. of days in parabiosis	No. of days Hypophysectomized	Days castrated or Cryptorchid	Organ weights (Grams)					Histological			
				Testes	Seminal Vesicles	Ventral Prostate	Dorsal Prostate	Adrenals	Testis Tubules	Interstitial cells	Seminal Vesicles	Ventral Prostate
Hypophysectomized Male (h)+Castrated Male (c)												
17h	11	13	—	0.84	.208	.041	.081	.017	−	−	−	−
17c	11	—	11	—	.114	.044	.067	.035	−	−	−	−
4h	13	15	—	1.26	.108	.044	.050	.011	++	++	−	−
4c	13	—	13	—	.112	.032	.050	.030	−	−	−	−
72h	14	16	—	2.11	.314	.136	.143	.015	+++	+++	++	++
72c	14	—	14	—	.167	.071	.097	.043	−	−	−	−
1h	19	21	—	1.83	.110	.048	.050	.011	+++	+++	+	++
1c	19	—	19	—	.096	.019	.055	.042	−	−	−	−
65h	20	22	—	0.99	.126	.190	.080	.012	+	+	−	−
65c	20	—	20	—	.077	.038	.044	.040	−	−	−	−
84h	49	21	—	2.39	1.586	.367	.311	.013	++++	++++	++++	++++
84c	49	—	21	—	.069	.021	.045	.030	−	−	−	−
84nc	Litter control			2.53	.897	.289	.188	.023	++++	++++	++++	++++
91h	48	41	—	1.40*	.591	.101	.069	.011	++	++++	++++	++++
91c	48	—	20	—	.062	.021	.032	.034	−	−	−	−
91nc	Litter control			2.64	.674	.245	.125	.021	++++	++++	++++	++++
Hypophysectomized Male (h)+Cryptorchid Male (cr)												
39h	14	17	—	0.82	.125	.036	.053	.010	−	−	−	−
39cr	14	—	28	0.66	.336	.170	.091	.031	−	++++	++++	++++
8h	15	15	—	1.37	.112	.066	.066	.012	+	+	−	+
8cr	15	—	15	0.78	.714	.217	.159	.029	−	++++	++++	++++
51h	21	23	—	0.35	.050	.024	.022	.014	−	−	−	−
51cr	21	—	71	0.59	.267	.204	.085	.033	−	++++	++++	++++
52h	21	23	—	0.39	.055	.015	.024	.012	−	−	−	−
52cr	21	—	71	0.79	.683	.173	.120	.030	−	++++	++++	++++
Hypophysectomized Male (h)+Normal Male (n)												
69h	15	17	—	1.01	.121	.039	.048	.014	++	+	−	−
69n	15	—	—	1.76	.173	.075	.068	.047	++++	+++	+	++
67h	20	22	—	1.07	.081	.031	.045	.012	++	++	−	−
67n	20	—	—	2.21	.689	.338	.186	.038	++++	++++	++++	++++
66h	20	22	—	0.61	.081	.050	.037	.011	−	−	−	−
66n	20	—	—	3.04	1.131	.346	.213	.040	++++	++++	++++	++++
13h	24	24	—	0.82	.167	.062	.077	.013	−	−	−	−
13n	24	—	—	1.40	.758	.204	.214	.037	+	++	++	++

— Indicates lack of stimulation.
+ Indicates various degrees of stimulation, ranging from slight stimulation (+) to normal (++++).
* Doubled weight of right testis; left testis removed at start of experiment.

whether the body cavities were in open connection (celio-anastomosis) or in closed connection. We have made observations which confirm those of Hill and which further indicate that in adult animals hormonal exchange adequate to induce demonstrable effects does not occur until 7 to 12 days after parabiosis. Therefore, in those pairs in which hypophysectomy preceded or was per-

formed concurrently with parabiosis, a certain amount of degeneration in the genital organs was to be expected, as the pairs were sacrificed before complete or partial restoration could have been effected.

Hypophysectomized male + castrated male. The testes of all but one of the hypophysectomized animals showed varying degrees of stimulation as determined by their weights. When gonadokinetic effects were produced, they were apparent both in the interstitial cells and in the seminiferous epithelium. In fact there was a striking parallelism between the degree of stimulation of germinal and interstitial cells. In those animals which were hypophysectomized before the time of parabiosis, there was not a strict correlation between the histological appearance of the accessory organs and the interstitial cells of the testis. That temporal and threshold factors were probably involved in this discrepancy is illustrated by the results obtained in the animals hypophysectomized subsequent to the time of parabiosis.

The rats 84h, 84c, and 84nc were littermates. When they were 58 days old, two of them were united parabiotically and one was kept as a normal control. After the animals had been in parabiosis for 28 days (age 86 days), one of them (84h) was hypophysectomized, and the other (84c) was castrated. Three weeks later (age 107 days) the pair was sacrificed. That the testes of the hypophysectomized animal were producing an abundance of male hormone and that the castrated partner was receiving little or none of this secretion was indicated by the condition of the scrota in the two animals (fig. 1). The genital organs of the castrated partner were atrophic (fig. 3), while those of the hypophysectomized animal were macroscopically (fig. 2) and microscopically comparable with those of the normal control. The accessory organs of the hypophysectomized parabiont weighed more than the corresponding organs of the control, indicating a high degree of interstitial cell activity.

A second group of littermates was aged 59 days at the beginning of the experiment when two of them were joined in parabiosis; the third rat served as a normal control (91nc). One testis was removed from the animal which was subsequently to be hypophysectomized. A week after the time of parabiosis, the semigonadectomized animal (91h) was hypophysectomized; its partner remained normal. Up to this time the scrota of both animals had appeared normal. As far as could be determined by palpation, the remaining testis of 91h and the testes of its twin were normal also. Shortly following the ablation of the pituitary in 91h, the scrotum began to regress until at the end of 3 weeks it had almost disappeared; the testis was flabby and could be palpated only with difficulty. It was evident that this hypophysectomized animal was receiving little, if any, gonad-stimulating hormone from its normal twin. At this time (the animals were then 89 days old) the normal partner (91c) was castrated. The effects observed in the hypophysectomized animal after its partner had been castrated were striking. Within a few days the scrotum began to become red and turgescent, and the testis came to occupy a scrotal position. In the castrated animal, on the other hand, the scrotum began to lose color and to regress. Scrotal turgescence and vascularity and growth of the testis in the hypophysectomized rat visibly progressed until the

time of autopsy, 20 days after castration (the animals were then 107 days old). Figure 4 shows the condition of the scrota of this pair of animals at the time of autopsy. All the genital organs of the hypophysectomized parabiont weighed less than did corresponding organs of the littermate. However, the macroscopic and microscopic appearance of the genital organs approached that of the normal condition. (Compare figures 5 and 6 and figures 7, 8, 9, 10, with 11, 12, 13 and 14). The histological appearance of the seminal vesicles

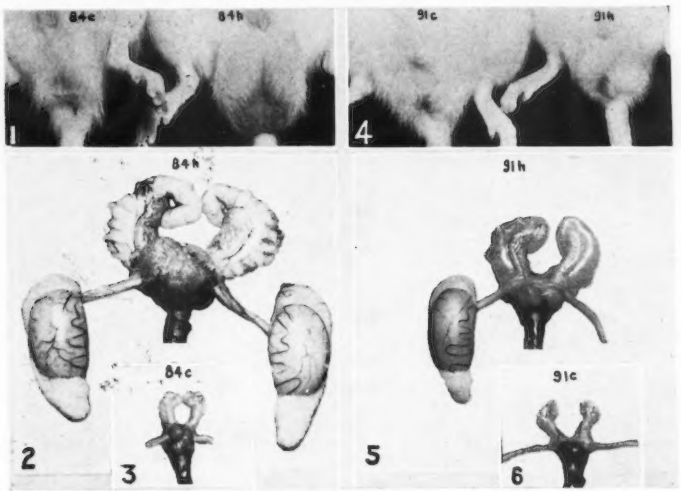

PLATE 1. (Figures 2, 3, 5, and 6 were all taken at the same magnification).
Fig. 1. Observe large scrotum of hypophysectomized partner (84h) and regressed scrotum of its castrated partner (84c).
Fig. 2. Genital organs of hypophysectomized partner, 84h.
Fig. 3. Accessory sexual organs of castrated partner, 84c.
Fig. 4. Note turgescence of scrotum of the hypophysectomized partner (91h) and regression of scrotum in the castrated partner (91c). Three weeks before this photograph was taken, 91c had not been castrated, and the scrotum of 91h at that time resembled that of the castrated animal as seen here. The outline of the right testis and epididymis of 91h is visible; the left testis had been removed previously.
Fig. 5. Genital organs of hypophysectomized partner, 91h.
Fig. 6. Accessory sexual organs of castrated partner, 91c.

and prostate of the castrated control is shown in figures 15 and 16.

Hypophysectomized male + cryptorchid male. Only one of the hypophysectomized rats in this series showed signs of gonadal stimulation. Again, however, it was noted that the stimulation, though slight, affected both interstitial and germinal cells. It seems very probable that if the hypophyses of the animals which had been cryptorchid for 71 days (50 days at the time of parabiosis) had become hyperactive, some effect would have been produced in the hypophysectomized partners.

Hypophysectomized male + normal male. In two of the hypophysectomized animals gonadal stimulation was induced. This was not marked in either animal, but again in both instances the interstitial cells and the germinal cells were affected. It will be observed that the testes of two of the normal partners (69n and 13n) were not normal in weight or histological appearance. This may possibly be explained as a result of the distribution between two partners of an amount of hormone sufficient for the needs of only one.

DISCUSSION

The gonadal stimulation observed in some of the hypophysectomized parabionts must have been effected by secretion liberated by the hypophyses of

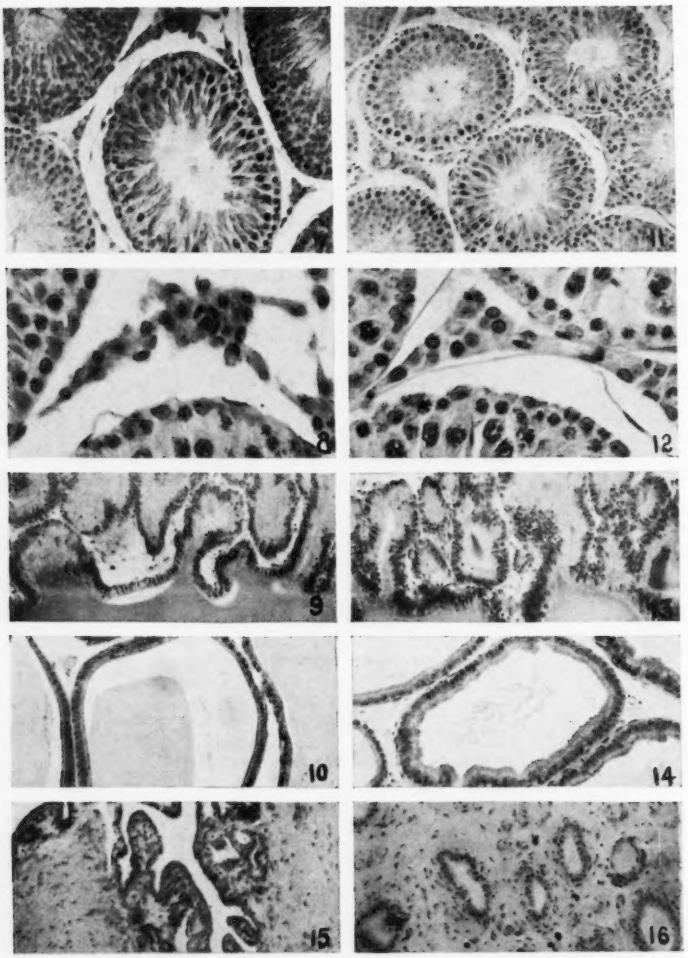

PLATE 2.

Fig. 7. Section of testis of normal control littermate, 91nc. ×125.
Fig. 8. Enlarged view of interstitial cells seen at top center of fig. 7. ×400.
Fig. 9. Section of seminal vesicle of normal control littermate, 91nc. ×125.
Fig. 10. Section of ventral prostate of normal control littermate, 91nc. ×125.
Fig. 11. Section of testis (see fig. 5) of hypophysectomized rat, 91h, which had been in parabiosis with a castrated partner. Note smaller tubular diameter as compared with that in fig. 7 and abundance of interstitial tissue. The portion of the tubule at the lower left corner shows disorganization of the seminiferous epithelium. ×125.
Fig. 12. Enlarged view of interstitial cells seen a little above and to the right of the center of fig. 11. Observe large round nuclei and abundant cytoplasm. ×400.
Fig. 13. Section of seminal vesicle of rat 91h. ×125.
Fig. 14. Section of ventral prostate of rat 91h. ×125.
Fig. 15. Section of seminal vesicle of castrated partner 91c. ×125.
Fig. 16. Section of ventral prostate of castrated partner 91c. ×125.

the partners. As has been pointed out the quantitative response of the testes might have been influenced by various factors other than hormonal stimulation, such as time and the extent of vascular anastomosis. In view of the rigidly uniform techniques employed in this study, however, it seems reasonably certain that in all pairs which had been in parabiosis for 15 days or more, the quantitative response of the testes of the hypophysectomized test rat roughly indicated the relative gonadokinetic potency of the anterior lobe of the pituitary gland of the partner. From the results its seems evident that the hypophysis of the castrated animal produced relatively more gonad-stimulating hormone than did that of the cryptorchid or normal animal. That gonadectomy causes an increase in the gonad-stimulating capacity of the anterior lobe of the hypophysis has already been demonstrated by studies on implantation in rats (14, 15). The hypophyses of cryptorchid rats, whose testes had been abdominal in position for as long as 71 days, failed to induce a greater sex-stimulating effect than did the hypophyses of normal rats. An increase in gonadokinetic potency of the anterior lobe of the pituitary which has been said to occur following cryptorchidism in rats (10, 15, 16) either did not occur in our animals, which seems probable, or was not demonstrable by our technique.

Regarding the qualitative effects on the gonads of the hypophysectomized parabionts, the results, when stimulation occurred, were in every instance the same. Whether the donor twin was castrated, cryptorchid, or normal, the testes of the hypophysectomized partner showed parallel stimulation of the germinal and interstitial cells. In no instance was there observed a purely gametokinetic effect. This fact not only demonstrates that the hypophysis of the castrated animal produces and liberates large amounts of luteinizing or interstitial cell-stimulating hormone, but also lends support to the idea that the anterior lobe of the hypophysis of the male rat elaborates but one gonadokinetic hormone.

Though the anterior lobe of the pituitary did produce two gonad-stimulating hormones, one gametokinetic and the other interstitial cell-stimulating, it would be difficult to believe that a purely gametokinetic effect could be demonstrated in the male rat, an animal in which the scrotum is essential to normal spermatogenesis. Proper scrotal function is probably controlled entirely by the male sex hormone (17) which is presumably produced by the interstitial cells of the testis. By inference, therefore, it seems obvious that spermatogenesis in the rat can occur only if the interstitial cells are stimulated to produce male hormone or if male hormone is supplied artificially. Since previous studies (17) have shown that spermatogenesis can be maintained in the absence of hypophyseal secretion by means of male hormone alone, it seems reasonable to suppose that but one hypophyseal hormone acting on the interstitial cells of the testis would be required for the functioning of the testis both as an androgenic and a reproductive organ. The implication, of course, would be that spermatogenesis in the rat is not directly dependent on hypophyseal stimulation.

SUMMARY AND CONCLUSIONS

Varying degrees of stimulation occurred in the testes of *a)* 5 of 6 hypophysectomized rats joined parabiotically with castrated partners; *b)* 1 of 4 hy-

pophysectomized rats joined with cryptorchid partners; and *c)* 2 of 4 hypophysectomized rats joined with normal partners.

Greater stimulation of the testes occurred in hypophysectomized parabionts when the partners were castrates than when the partners were normal or cryptorchid. The testes of hypophysectomized rats joined with cryptorchid or normal animals were but slightly stimulated.

The results indicated *a)* that the hypophyses of castrated rats produced or liberated a greater amount of gonad-stimulating hormone than normal hypophyses and *b)* that the hypophyses of cryptorchid rats did not produce or liberate more gonad-stimulating hormone than did normal glands.

A purely gametokinetic effect which has been said to occur in hypophysectomized female rats joined with castrated partners did not occur in hypophysectomized male rats joined with castrated male twins. In all instances in which gonadokinetic effects occurred in the testes of hypophysectomized rats, whether the partner were castrated, cryptorchid, or normal, it was observed that there was a parallel stimulation of the germinal and interstitial cells. The histological appearance of the interstitial cells proved to be a more reliable criterion of the degree of interstitial cell activation than did the weight or microscopic appearance of the accessory sexual organs.

The results are discussed with reference to the production by the anterior lobe of the pituitary gland of the male rat of one or two gonadotropic hormones. This and previous studies have provided presumptive evidence that in the male rat but one hypophyseal gonadokinetic hormone is required for the functioning of the testis both as an androgenic and a reproductive organ.

Grateful acknowledgment is made to Mr. W. Kenneth Cuyler for the photomicrographs.

REFERENCES

1. Hamburger, C.: Acta path. et microbiol. Scandinav. (suppl.) **17:** 1. 1933.
2. Hellbaum, A. A.: Proc. Soc. Exper. Biol. & Med. **30:** 641. 1933.
3. Hellbaum, A. A.: Anat. Rec. **63:** 147. 1935.
4. Smith, P. E., and E. T. Engle. J. Pediat. **5:** 163. 1934.
5. Witschi, E., and W. T. Levine: Proc. Soc. Exper. Biol. & Med. **32:** 101. 1934.
6. Møller-Christensen, E.: Acta path. et microbiol. Scandinav. (suppl.) **10:** 296. 1933.
7. Evans, H. M., M. E. Simpson and R. I. Pencharz: Proc. Soc. Exper. Biol. & Med. **32:** 1048. 1933.
8. Lipschütz, A., and G. Villagran: Compt. rend. Soc. de biol. **121:** 203. 1936.
9. Møller-Christensen, E.: Studien über das Zusammenspiel von Hypophysen- und Ovarialhormonen, inbesondere im Lichte von Parabioseversuchen. Acta path. et microbiol. Scandinav. (suppl.), **22:** 1. 1935.
10. DuShane, G. P., W. T. Levine, C. A. Pfeiffer, and E. Witshi. Proc. Soc. Exper. Biol. & Med. **33:** 339. 1935.
11. Thompson, K. W.: Endocrinology. **16:** 357. 1932.
12. Walker, G.: Bull. Johns Hopkins Hosp. **21:** 182. 1910.
13. Hill, R. T.: Proc. Soc. Exper. Biol. & Med. **28:** 866. 1931.
14. Engle, E. T.: Am. J. Physiol. **88:** 101. 1929.
15. Evans, H. N., and M. E. Simpson: Am. J. Physiol. **89:** 371. 1929.
16. Martins, T.: Compt. rend. Soc. de biol. **105:** 789. 1930.
17. Cutuly, E., D. R. McCullagh and E. C. Cutuly: Am. J. Physiol. In Press.

THE INTERRELATIONSHIP OF THE PITUITARY SEX HORMONES IN OVULATION, CORPUS LUTEUM FORMATION, AND CORPUS LUTEUM SECRETION IN THE HYPOPHYSECTOMIZED RABBIT[1]

M. A. FOSTER,[2] RUTH CALDWELL FOSTER AND F. L. HISAW
From the Biological Laboratories, Harvard University
CAMBRIDGE, MASSACHUSETTS
and The University of Wisconsin Medical School
MADISON, WISCONSIN

Previous studies carried on in these laboratories have indicated that follicular development in the rabbit is probably brought about by the follicle-stimulating hormone (FSH) of the pituitary; that ovulation is closely associated with, if not the result of, an augmentation reaction which obtains when small amounts of the luteinizing hormone (LH) are introduced into the blood stream with FSH in a fairly definite ratio; and that corpus luteum secretion is regulated by these hormones (1). Since the pituitary represented an unknown variable in these experiments on immature rabbits, it seemed desirable to rule out its influence by hypophysectomy. Inasmuch as the immature rabbit does not usually survive this operation satisfactorily, it became necessary to use the adult.

The adult female rabbit has been successfully hypophysectomized by several workers who have reported progressive gonadal regression resulting in complete atrophy of the large follicles in 2 weeks (2), a corresponding decrease in the size of the corpora lutea (3), atrophy of other endocrine glands, associated with certain metabolic changes, loss of hair and generalized cachexia.

The rapid regression of the follicular apparatus makes the hypophysectomized rabbit ideal for studying the induction of follicular development through the various stages of growth, preovulatory swelling, ovulation, and luteinization. It is also possible under these conditions to determine the functional capacity of the corpora lutea.

This has been our main objective, although in the execution of this program supplementary data have accumulated on the normal involutionary changes occurring in the genital tract from the time of hypophysectomy to the 130th post-operative day. In addition, studies have been made on the effects of FSH and LH preparations on fasting blood sugar levels of the hypophysectomized rabbit.

[1] Aided by a Grant from the National Research Council, Committee on Problems of Sex.
[2] General Education Board Fellow in Endocrinology.

Adult female rabbits which had been isolated were used throughout these experiments. The technique of hypophysectomy was the intraorbital approach described by Firor (4) and all animals were checked at autopsy by gross and histological examinations of sellar contents. Blood sugar determinations were made according to the method of Folin and Wu and extracts were prepared by the methods described by Fevold and Hisaw (5).

EARLY EFFECTS OF HYPOPHYSECTOMY

Normally, the earliest changes noted after hypophysectomy were diuresis and blanching of the vulva. Diuresis occurred within a few hours. Blanching was accompanied by a progressive atrophy of the vulva which began the first day and was quite marked by the fifth day. In many animals shedding also occurred after 10 to 14 days. As was pointed out by Cope and Marks (6) and others there is a fall in the fasting blood sugar level, so that frequently a low carbohydrate reserve resulted in prostration and death in a few hours. The average sugar level after a few hours fasting (6 to 8) was usually 60 to 70 mg., while in those animals near prostration it was frequently below 40 mg.

Histological changes in the genital tract. Fourteen rabbits were autopsied at intervals as follows: 24, 36, 45 and 86 hours; 6, 17, 20, 22, 30, 55, 83, 95 and 130 days after hypophysectomy. Histological sections were made of both ovaries and uteri (table 1). Those killed before 6 days included animals that were hypophysectomized during estrous and 1 to 2 days after mating. In this group it was possible to study the changes which occur in the graafian follicles, corpora lutea, and uteri immediately after hypophysectomy.

The first structures to show significant changes after operation were the large follicles. These began to show signs of atresia as early as 24 hours, as indicated by a clumping of the chromatin and achromatolysis of the granulosa cells. Later the thecal cells underwent hypertrophy, the ovum degenerated, and by the sixth day few if any of the large follicles remained. Beyond this period no follicles larger than 0.5 mm. were observed (fig. 1).

When corpora lutea were present, a process of degeneration began within 36 hours and ended, in so far as physiological activity of the corpora was concerned, by the fifth or sixth day. The histological changes associated with loss of function (based on histology of the uterus) were confined almost entirely to the lutein cells themselves (fig. 2 and 5), although there was an apparent overgrowth of the fibrous connective tissue framework which enmeshes the lutein cells. Early changes in the lutein cells showed only vacuolar degeneration of the cytoplasm, but this was followed by a degeneration of the nucleus. The nuclei were surrounded by large fat vacuoles and took more of the acid dyes (fig. 2). Later, the entire cell was represented by a large fat vacuole. Degeneration of the lutein cells resulted in a decrease in gross size of the corpora lutea. Associated with this luteal involution there occurred a corresponding involution of the glands of the pseudopregnant uterus (fig. 3 and 6). This atrophic process in the uterus began in the basal part of the glands and passed upward. At 86 hours after hypophysectomy there was a marked regression from a +4 to a +1 or +2 reaction; by 6

days it was complete, and the uteri resembled those of castrated animals.

Following the sixth day after hypophysectomy there was a progressive decrease in the number of both small and medium follicles and oöcytes, with eventually a vacuolar degeneration of the interstitial cells, while at the same time the uterus atrophied to a small and compact structure very similar to

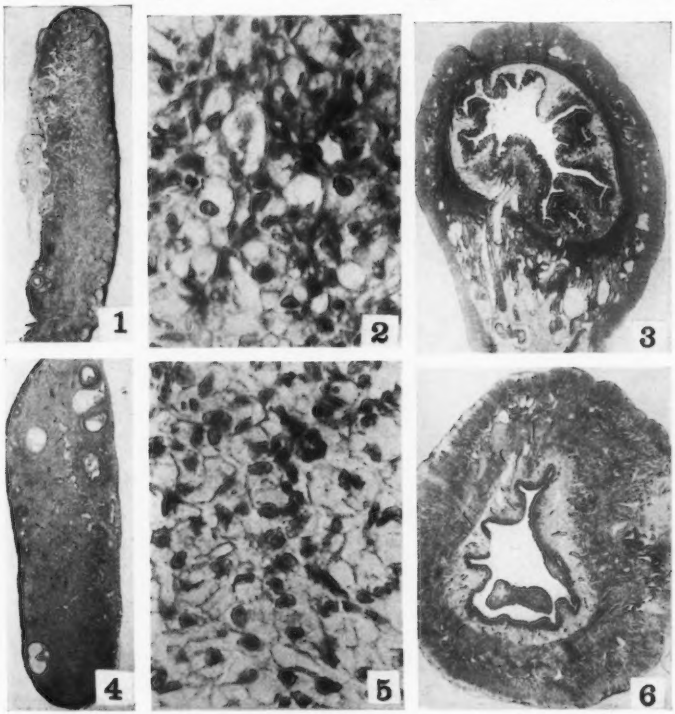

Fig. 1-6. *1*. Ovary of untreated rabbit 27, 17 days after hypophysectomy. Solid interstitial tissue and a few small follicles. ×4. *2*. Section of a corpus luteum 86 hr. after hypophysectomy. Note the large cytoplasmic vacuoles and the achromatolysis of the nuclei (light staining ones are eosinophilic). No. 33. ×710. *3*. Uterus of the same animal shown in *2*. Note the widening of the lumen and shallow coiled glands. Compare with fig. *10*, *11* and *12*. Probably a +1 reaction from corpora which grossly appear to be normal. No. 33. ×8. *4*. Ovary of hypophysectomized rabbit which received 1 R.U. of purified FSH extract daily from the 26th to the 35th days after hypophysectomy. Note the atretic ova and degenerate follicular apparatus. See fig. *15* for the uterus of this animal and fig. *1* for the control ovary. No. 1. ×4. *5*. Section for the peripheral portion of a corpus luteum 5 days after hypophysectomy. Note the pycnotic nuclei, the vacuolated cytoplasm and the invasion of leucocytes. Compare with fig. *7* for a normal corpus luteum, and with fig. *2* for an earlier regressive stage. No. 607. ×710. *6*. Uterus of 607 showing almost complete regression. See fig. *5* for corpora lutea. ×8.

that found in the immature animal. Atresia of the medium and small follicles was characterized by a progressive vacuolation and achromatolysis of the granulosa cells, fragmentation of the ovum, and as these structures degenerated the follicle was encroached upon by thecal cells which gradually increased in number to form a thick ring which eventually replaced the follicle as a whorl of thecal cells. All stages in this process were observed from 6 to 30 days after hypophysectomy, but the number of small follicles with antra showed a gradual decrease until very few were seen by 30 days.

At 30 days after hypophysectomy, the interstitial cells were found to

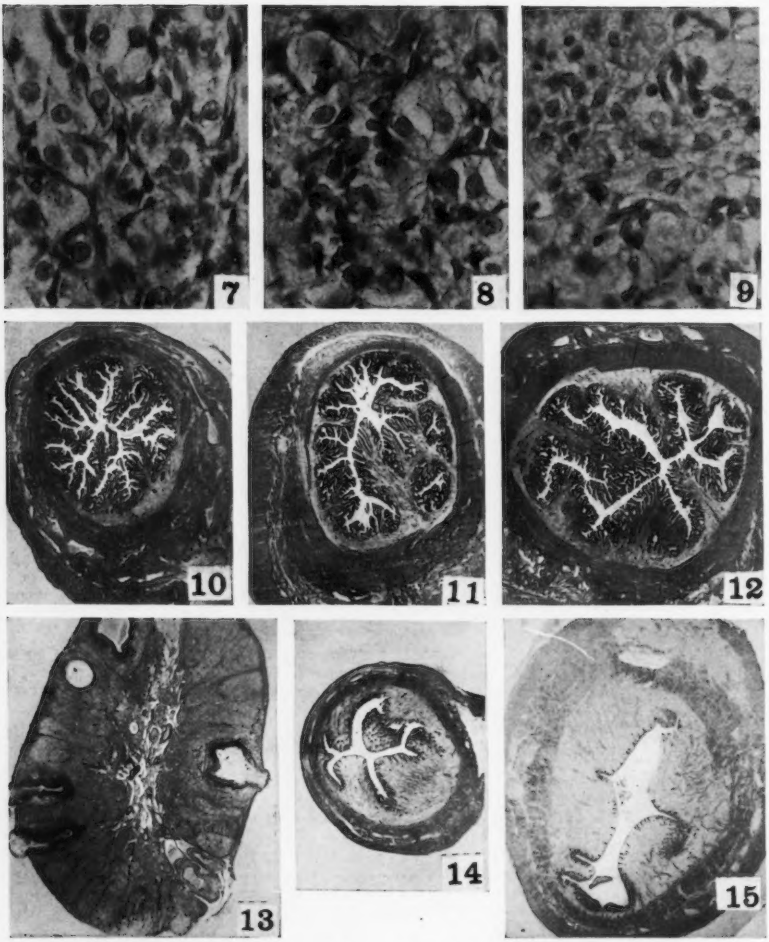

Fig. 7-15. *7.* Following experimental ovulation as shown in *13* this animal was injected for 4 days with a mixture of 2 R.U. FSH extract and 30 mg. eq. LH which resulted in the formation of normal corpora and a pseudopregnant uterus (fig. *10*). This is essentially a normal corpus luteum. No. 24. ×555. *8.* A section of a corpus luteum developed by 50 mg. eq. of LH daily for 3 days following experimental ovulation. The corpora were grossly normal and the uterus pseudopregnant (fig. *11*). Note the large vacuoles in the cytoplasm. No. 608. ×555. *9.* The connective tissue mass which develops at the site of ruptured follicles after treatment with FSH extracts. This structure has no secretory capacity as shown by the uterus (fig. *14*). The cytoplasmic material stains like hyaline. No. 18. ×555. *10.* Pseudopregnant uterus associated with the experimentally produced corpus luteum shown in fig. 7. No. 24. ×6. *11.* Pseudopregnant uterus corresponding to fig. *8*. Compare the degree of progestational change with that developed after administration of FSH and LH combinations, fig. *10* and *12*. No. 608. ×6. *12.* Pseudopregnant uterus developed experimentally 18 days after hypophysectomy by the administration of a combination containing 4 R.U. of FSH and 100 mg. eq. of LH daily for 3 days after experimental ovulation. Compare with fig. *10*, *11* and *14*. No. 25. ×6. *13.* Ovulation points 16 days after hypophysectomy. This animal was treated as follows: 1) 3 R.U. of FSH extract daily on the 9th to the 14th days; 2) 6 R.U. FSH and 10 mg. eq. of LH intravenously on the 15th day. Result, 13 ovulation points. Note the absence of early luteinization of granulosa so commonly observed in normal follicles at the time of ovulation. No. 29. ×8. *14.* Following experimental ovulation 4 R.U. of FSH was administered daily for 4 days. No corpora were developed at the ovulation points, and the uterus was in a low estrous state. See fig. *9* for the structure of the connective tissue whorls which developed at the rupture points. Compare with fig. *12* which received the same dosage of FSH combined with LH. No. 19. ×6. *15.* Estrous uterus 35 days after hypophysectomy obtained by the administration of 1 R.U. of FSH daily for 9 days. See fig. *4* for ovary of same animal. No. 1. ×6.

contain numerous cytoplasmic vacuoles which gradually coalesced, surrounding the nucleus which soon degenerated. This residual vacuole gradually disappeared, and by 55 days few interstitial cells remained. The ovaries at this time were very small, containing a few primordial follicles and oöcytes, while the medullary portion of the ovary, which in the adult is filled with interstitial cells, contained densely packed stroma which had an embryonic appearance due to the highly chromatic nuclei. From 55 to 90 days there was little change. The medullary part of the ovary filled in with dense stroma, while the cortex usually contained a few primordial follicles and oöcytes.

The uterus atrophied after hypophysectomy to a castrate-like condition and by the end of the second month attained an appearance resembling an infantile organ. The lumen became small and slitlike, and was lined with low cuboid epithelium.

EFFECTS OF THE TWO SEX HORMONES UPON FASTING BLOOD SUGAR LEVELS IN THE HYPOPHYSECTOMIZED RABBIT

In the course of this work on hypophysectomized rabbits, it soon became apparent that one of the easiest ways of determining the success of an operation for removal of the pituitary was the lowered blood sugar level after a brief fasting period. This hypoglycemia usually developed by about the fifth day following hypophysectomy, and after this time it became necessary to exercise caution in exposing these animals to a fast of more than 8 hours. Since Cope and Marks (6) have demonstrated that the administration of crude hypophyseal extracts (beef) to hypophysectomized rabbits restored the fasting blood sugar levels to normal and enabled them to fast 24 hours or longer, we were interested in determining the role of FSH and LH extracts in this capacity. At varying intervals after hypophysectomy follicle-stimulating extract was prepared from horse and hog pituitaries and administered over periods of 5 to 10 days, to 20 rabbits in dosages of 3 to 5 R.U. daily (0.1 to 1.0 gm. equivalent). It was found that there were no appreciable changes in the fasting blood sugars from those of untreated controls. For example the normal blood sugar of rabbit 10 after 5 hours' fasting was: 60 mg. per cent (7th day); 45 mg. (9th day); 61 mg. (11th day). This same animal when given FSH, starting on the 14th day, had 55 mg. (16th day) and 45 mg. (19th day). A similar result was obtained with LH prepared from hog pituitaries when administered in small doses (20 mg. equivalent daily).

EFFECTIVENESS OF FSH IN PREVENTING FOLLICULAR ATRESIA IN THE HYPOPHYSECTOMIZED RABBIT

Hypophysectomized animals treated with FSH (2 to 3 R. U. daily) did not show follicular atresia when injections were begun within 2 or 3 days after hypophysectomy. Ovaries and uteri remained normal as shown by large follicles and hyperemic uteri. When injections were begun later than 3 days the degree of atresia depended upon the time elapsing between hypophysectomy and the beginning of injections (fig. 4). However, on comparing treated animals with hypophysectomized controls, 5 or more days of treatment demonstrated a certain amount of repair regardless of the interval of

regression. Delayed injections manifested a definite tendency toward preventing vacuolar degenerative changes of the granulosa cells, and a reduction in the number of follicles undergoing atresia. Since the hypophysectomized animal at 30 days has only a few small follicles remaining, repair, if possible at all, would require much longer treatment.

As long as antra-containing follicles remained in the ovaries, FSH caused

TABLE 1. UNTREATED HYPOPHYSECTOMIZED RABBITS.

Animal	Autopsy	Uterus	Ovary
K37	36 hr.	++ or +++; mitoses present	Early degeneration of corpora and large follicles.
32	45 hr.	++; basal glands degenerated	Same
33	86 hr.	+ or ++; same	Corpora small, vacuoles in lutein cells. No large follicles.
13	6 days	Low estrus	Follicles (0.3–0.5 mm.) remain; beginning atresia of these.
27	17 days	Small and atrophic	Follicles smaller; more advanced atresia.
4	20 days	Same	Vacuoles appear in interstitial cells.
2	22 days	Same	Same; only a few small follicles remain.
12	30 days	Same	Same; great reduction in size.
18	30 days	Same	Stroma cells more prominent.
1309	55 days	Immature type	No interstitial cells; only stroma and a few small follicles.
30	83 days	Same	Same
599	95 days	Same	Medulla, stroma cells only. Cortex, few oöcytes only.
544	130 days	Same	Few follicles and oöcytes still present.

an enlargement of them with an increased estrin output. One animal treated 26 days after hypophysectomy over a period of 8 days was brought into a full estrous condition with large follicles and enlarged hyperemic uteri, but the follicles were cystic and could not be ovulated (fig. 4 and 15). No follicular enlargement with hyperemia of the secondary sex organs was elicited by similar treatment in any animal hypophysectomized for more than 30 days.

EXPERIMENTAL OVULATION IN THE HYPOPHYSECTOMIZED RABBIT

The atrophic changes in the reproductive system which occurred within 3 to 5 days after hypophysectomy indicated that the loss of circulating pituitary hormones was rapid and complete. Atresia of the large follicles at this time had progressed to the point where they could no longer be ovulated by the

intravenous injection of large doses of AP extracts. In this connection White and Leonard (7) showed that within an hour after hypophysectomy the minimal ovulating dosage (M.O.D.) was 1/6 to ½ greater than that required before operation. It was necessary therefore to produce new follicles experimentally before ovulation could be obtained.

In the present investigation a standard period of 5 days treatment was adopted for follicular development. This was based upon the time required to induce follicular growth in an immature rabbit, and the fact that in the normal adult rabbit there is a new group of follicles about every 8 days (8). The extracts used were purified and semi-purified FSH prepared from horse and hog pituitary powder. The pituitary preparations used for intravenous injections to produce ovulation after preliminary follicular growth was a mixture of FSH and LH, about 50 parts FSH to 1 of LH. Treatment was begun at varying intervals from 6 to 35 days after hypophysectomy and consisted of: 1) subcutaneous injection of 3 to 6 R.U. of FSH daily for 5 days for follicular development; 2) an intravenous injection of 0.3 to 0.5 gm. eq. of FSH (2 to 10 R.U.) augmented by 5 to 10 mg. eq. of LH 8 to 10 hours after the last injection of FSH. Ovulation occurred 18 to 24 hours later.

The results on a total of 20 animals treated 6 to 30 days postoperatively showed that 16 of the group had a total of 156 ovulation points, (average 9.7 each) and that the number of ovulation points was closely related to the length of time which elapsed after hypophysectomy, before treatment was begun. The amount of atresia is of course related to the length of this time interval and determines the degree of ovarian reactivity. Beginning with an average of 14 ovulation points when treatment was begun on the sixth day, the average steadily dropped until the reaction faded completely between 2 and 3 weeks. This suggests that the remaining antra-containing follicles large enough to respond at this time could not be developed by FSH in 5 days to a point at which they could be ovulated. Beyond this period the situation was similar to that found in the immature rabbit about 11 weeks of age. A few small follicles were present, several containing antra, which were capable of being stimulated but did not seem capable of ovulation.

A series of 6 animals was treated, as described above, a second time 2 to 3 weeks after a previous experimental ovulation to determine whether a second group of follicles could be developed by a 5-day treatment of FSH. The results indicated that once the available follicles have been utilized experimentally they are not replaced in the absence of the pituitary. Of the 6 animals treated, there was a questionable ovulation point in one.

Two series of 3 animals each were treated as above for follicular development beginning about the ninth post-operative day. Purified hog FSH (1.0 gm. or 4-5 R.U.) was used for the intravenous injections in 1 group and sheep LH for the other. Neither series showed any ovulation points. This indicates that FSH or LH alone are probably unfavorable for inducing ovulation in the hypophysectomized rabbit.

Effect of FSH on the development and functional activity of lutein tissue following experimental ovulation in the hypophysectomized rabbit. Ovulation

was induced as previously described, but instead of allowing regression to occur without development of corpora lutea and pseudopregnancy, FSH was injected subcutaneously immediately, (table 2). Four animals in this group treated with FSH for 3 to 4 days (3 on the same dosage used for follicular growth) showed estrous uteri with demonstrable lutein tissue in only 1 case. Rabbit 17 treated daily after ovulating with 8 R.U. of FSH containing traces of LH showed incompletely developed corpora lutea and several follicles of varying size. The smaller follicles showed mild atretic changes. Rabbit 19 treated daily with 2 R.U. of a purified FSH preparation showed a connective

TABLE 2. THE EFFECT OF FSH, LH, AND MIXTURES OF THE TWO UPON THE DEVELOPMENT OF A FUNCTIONAL CORPUS LUTEUM WHEN INJECTED IMMEDIATELY AFTER EXPERIMENTAL OVULATION HAS BEEN INDUCED IN THE HYPOPHYSECTOMIZED RABBIT.

Animal	Ovulation Points	Treatment	Corpora lutea	Uteri
		Group 1, treated with FSH		
17	18	8 R.U.×3 days	Lutein rings, or cysts	Estrous
19	8	4 R.U. purified×4 days	Fibrosed points	Same
20	2	Same	Same	Same
596	5	Same	Same	Same
		Group 2, treated with LH		
15	9	200 mg. eq. 8×3 days	Solid corpora	++ pseudo-pregnancy
22	10	50 mg. eq. 7×3 days	None	Resting
29	13	200 mg. eq. 7×2 days	Loosely formed	++ pseudo-pregnancy
608	18	50 mg. eq. 8×3 days	Solid corpora	++++
		Group 3, treated with mixtures of FSH and LH		
24	13	4 R.U.+20 mg. eq.×4 days	Solid corpora	++++
25	15	4 R.U.+100 mg. eq.×4 days	Same	++++
607	13	4 R.U.+50 mg. eq.×4 days	Same	++++

tissue framework at the rupture points, but the interstices contained no lutein cells, simply an interwoven connective tissue mass which was apparently derived from and connected with the theca externa (fig. 9 and 14). A few small, mildly atretic follicles and a considerable amount of interstitial tissue were present. The ovaries of the other 2 animals were identical with those of rabbit 19 athough they had been given 4 and 8 R.U. of FSH respectively.

Effect of LH on the development and functional activity of lutein tissue following experimental ovulation in the hypophysectomized rabbit. Immediately following ovulation LH (sheep) was administered subcutaneously in varying doses for 2 to 3 days. On the fourth or fifth day uterine biopsies were removed and sections made of both ovaries and uteri (table 2). Three of the 4 animals showed corpora lutea and pseudopregnant uteri (fig. 8 and 11). Rabbit 15, treated with 4 times the dosage administered to rabbit 608, perhaps

should have had a more active secretion of corpus luteum hormone, but this animal died on the third day while the others were biopsied on the fifth day after LH treatment was begun. Histologically these corpora could not be distinguished from those in pregnant animals, making it apparent that LH not only stimulated the development of lutein cells, but also caused them to secrete the corpus luteum hormone.

Effect of a combination of FSH and LH on the development of lutein tissue and its functional activity after experimental ovulation. Three animals treated immediately following ovulation with a mixture of FSH and LH showed maximal pseudopregnancy reactions and normal corpora lutea. This suggests that the combination is better than LH alone for the development of lutein tissue and its functional activity, although it is true that treatment for ovulation in this group was begun somewhat sooner after hypophysectomy. In these animals the combination was varied as shown in table 2. The microscopic anatomy of the uteri showed a slight difference in the degree of glandular development, this being more pronounced in the animals receiving higher concentrations of LH (fig. 7, 10 and 12).

DISCUSSION

The altered reactivity of the follicular apparatus indicates that a progressive degenerative process begins soon after the pituitary is removed. Three to 5 days after hypophysectomy the existing follicles in the ovary lose their capacity to ovulate and subsequently there is a progressive decrease in the number which will respond to a 5-day treatment of FSH and are capable of being ovulated thereafter. This degenerative process involves not only follicles, but also the corpora lutea and eventually the interstitial cells leaving after 70 days or longer a small atrophic mass of connective tissue containing a few primordial follicles.

The prevention of involuntary changes in the follicular apparatus with FSH after hypophysectomy depends on the degree of destruction which has taken place. The large follicles degenerate early and may be quickly replaced by pituitary therapy as long as follicles of a medium size remain, but when these are lost as a result of involution it requires a longer time than 5 days to stimulate growth of the small follicles sufficiently for ovulation, hence the negative results for such experiments. Apparently small follicles which contain no antra are incapable of responding to the FSH treatment used in these experiments either by enlargement or estrin secretion.

A brief comparison of the ability of various preparations to induce ovulation, after preliminary follicular development with FSH extracts, is shown in the table below.

	FSH	FSH and trace LH	FSH and 100 mg. LH	LH alone
Juvenile rabbits	0	35	0	12–20
Hypophysectomized rabbits	0	12–18	not tested	0
Anestrous cats	4	20	9	15

Figures are the average number of ovulation points after intravenous injection of preparations. Each group represented by 5 to 20 animals.

The results for hypophysectomized rabbits agree with those for the cat and immature rabbit in that highly purified FSH extracts are unsatisfactory for ovulation, but the addition of traces of LH greatly enhances the ovulation response. Previous studies of the immature rabbit and cat showed that larger amounts of LH caused a definite reduction in the number of ovulation points. In addition, purified FSH preparations were found to have high minimal ovulation dosages in normal adult rabbits (80 mg. equivalent of sheep pituitary powder), but by adding 1 mg. equivalent of LH the dosage of FSH could be reduced to less than 20 mg. equivalent, a fourfold increase in activity by the addition of 1/25 of a M.O.D. of LH. The LH preparations per se were constant in their ability to elicit ovulation of a small number of follicles in the intact rabbit and cat after preliminary follicular growth, but they failed in the immature cat, immature monkey (Hisaw, unpublished observations) and the hypophysectomized rabbit—all of which have been ovulated successfully by using the combination.

In evaluating these results it must be apparent that such small quantities of LH as are necessary for optimal ovulation when combined with FSH will be present in many supposedly pure FSH preparations. Such partially purified preparations in moderate doses will cause only follicular enlargement in immature rats, but when the dosage is raised, cysts and corpora lutea appear. This type of preparation when injected intravenously will induce superovulation in immature rabbits which have been treated previously with FSH. After further purification, such preparations will not produce ovulation in sexually immature rabbits under the conditions mentioned above, even though their follicle-stimulating ability is the same, but when LH is added the capacity for inducing ovulation is restored. Therefore, in view of the difficulty in eliminating the last traces of LH one should be cautious in considering as pure an FSH preparation which gives ovulation unless it has been very carefully studied.

The augmentation reaction between FSH and LH seems to be the only phenomenon which correlates all the foregoing findings. It has been shown that LH may be aided in the ovulating reaction by small amounts of an FSH preparation (9) which may account for ovulation of normal animals by LH alone.

The failure of luteinization after experimental ovulation of hypophysectomized rabbits by a single intravenous injection of FSH plus a trace of LH is probably due to the lack of LH. This is suggested by the fact that after ovulation, administration of LH extracts alone or in combination with FSH leads to corpus luteum formation and pseudopregnancy. The amount of LH required for luteinization was much less when combined with FSH than when given alone, indicating a synergistic action between the two hormones in luteinization as well as in ovulation.

SUMMARY AND CONCLUSIONS

Hypophysectomy in the rabbit resulted in a progressive loss of function of the corpus luteum in about 5 days; large, medium and small follicles became

atretic and degenerated by the end of 2, 15 and 30 days respectively. Primary follicles persisted for as long as 90 to 100 days. Vacuolar degeneration of the intersititial cells began at 30 days and was completed by 55 days.

The tendency for the development of spontaneous hypoglycemia was not altered by either of the pituitary preparations employed in these experiments at the dosages used.

Follicle-stimulating extracts of the pituitary arrested follicular atresia and when injected within 3 days after hypophysectomy prevented it.

Following follicular growth, as a result of FSH treatment over a period of 5 days, ovulation was induced by an intravenous injection of FSH plus a trace of LH. The optimal mixture of the 2 pituitary preparations for ovulation seemed to be approximately 50 parts of FSH to 1 part of LH as based on weight of sheep pituitary powder. Though other combinations produced some ovulation it was found that when LH was increased a point was reached beyond which no ovulation occurred. The number of ovulation points varied directly with the interval between hypophysectomy and the first subcutaneous injection of FSH. When treatment was begun before 14 days, 1 to 18 ovulation points in each animal resulted, while after this time the ovaries rapidly failed to respond. Attempts at ovulation were unsuccessful when treatment was begun later than 23 days after hypophysectomy, although follicular growth and estrin secretion did occur.

Ovulation of hypophysectomized rabbits by a single intravenous injection of pituitary extracts was followed within a few days by atrophy of the granulosa with little or no development of lutein tissue. When ovulation was followed by treatment with FSH extracts alone the ruptured follicle failed to develop into lutein tissue and the uteri remained in an estrous condition. However, after administration of LH or a combination of the 2 hormones functional corpora lutea were formed as shown by a maximal pseudopregnancy reaction in the uterus.

REFERENCES

1. Foster, M. A., and H. L. Fevold: Anat. Rec. **61:** 18. 1933.
2. White, W. E.: Proc. Roy Soc., London, s.B. **114:** 64. 1933.
3. Smith, P. E., and W. E. White: J.A.M.A. **97:** 1861. 1931.
4. Firor, W.: Am. J. Physiol. **104:** 204. 1933.
5. Fevold, H. L., and F. L. Hisaw: Am. J. Physiol. **109:** 655. 1934.
6. Cope, O., and H. P. Marks: J. Physiol. **83:** 57. 1934.
7. White, W. E., and S. L. Leonard: Am. J. Physiol. **104:** 44. 1933.
8. Hill, Margaret, and W. E. White: J. Physiol. **80:** 174. 1933.
9. Foster, M. A., and F. L. Hisaw: Anat. Rec. **62:** 75. 1935. And unpublished data.

SOME FACTORS INFLUENCING THE VITALIZATION OF THE OVARIAN GRAFT AND THE PRODUCTION OF SEX HORMONES IN THE MALE RAT

CARROLL A. PFEIFFER[1]

From the Barbara Henry Research Laboratory of the New York Hospital and the Department of Medicine, Cornell University Medical College

NEW YORK CITY

Sex antagonism has been an interesting and strongly contested field of research. There is conclusive evidence that antagonism of the inductor systems in sex differentiation exists (1). However, it is now an accepted fact that after differentiation is complete, in the mammal at least, there is no direct antagonism between the gonads or their hormones. Any supposed antagonism has been explained by a suppressive action of the hormones upon the hypophysis (2), yet in some grafting experiments (3) it was observed that in certain groups of rats the male hormone and the ovarian grafts were both suppressed. In former experiments (4), using immature grafts from littermates or closely inbred strains, no suppression was noted.

This investigation is, therefore, an analysis of some possible factors responsible for the non-viability of the ovarian grafts and the suppression of the male hormone in the male host. An examination of the protocols of the previous experiment suggests three points: *1)* the ovarian grafts may have been from unrelated strains of rats; *2)* they contained lutein tissue at the time of grafting; *3)* many of the animals suffered to a certain extent from a respiratory infection, the so-called 'snuffles.' Since the present investigation shows that the respiratory infection is the important factor, this is further subdivided into temperature effect, toxicity, and inanition.

EXPERIMENTAL RESULTS

Nine castrate and 3 normal male rats were grafted with ovaries from 35 day old mice in the anterior chamber of the eye, in order to rule out any effect of non-relationship in grafting experiments. The grafts were removed after 14, 20, 25, 28, 30, 35, and 40 days, and in all but one case the characteristic ovarian stroma and connective tissue were readily recognized. After 35 to 40 days many leucocytes appeared and the resemblance to ovarian tissue was lost. Some grafts showed a definite vascularity which was not easily demonstrated in others. There was apparently no relationship between vascularity and the condition of the graft. There was no indication of any hormonal function of the grafts and the host accessory sexual organs were not affected. These grafts could not be considered as vitalized ovarian transplants.

[1] Henry Research Fellow in Medicine.

To study the effects of lutein tissue, 6 males 145 days of age received one-third of an ovary of a 14-month old female 10 days post-partum; 9 males 120 days of age received one-third of an ovary from females of the same age group; and 6 males 120 days old received 21 day old ovaries. At autopsy, after 45 to 60 days, the testes and accessory sexual organs were weighed and measured. The average for the group is recorded in table 1, *A*. Grafts, gonads and accessory sexual organs were prepared for histological study. However, 3 experimental and 2 control animals developed a respiratory infection, the so-called 'snuffles' and were isolated. The results from the animals with snuffles and 5 similar animals from a previous experiment (3) known to have snuffles, are shown in table 1, *B*. The definite parallelism of the experimental and

TABLE 1.

No. of Animals	Age at grafting	Age at autopsy	Type of graft	Body weight	Size of seminal vesicles	Wt. of seminal vesicles	Wt. of prostate	Wt. of testes	Condition of ovarian graft
	days	days	ovary	gm.	mm.	gm.	gm.	gm.	
A. Effects of ovarian grafts upon the male secondary sexual accessories									
6	145	205	postpartum	241	25.7× 8.8	1.161	0.661	1.943	Mainly stroma
2	controls	205		250	25.0× 8.0	1.130	0.480	1.100	
6	120	165	21 day	220	26.2× 9.1	1.070	0.648	2.354	Good follicular development
2	controls	165		220	27.0× 9.5	1.064	0.364	2.806	
6	120	165	120 day	231	28.0×10.3	1.128	0.606	2.286	Follicular development
1	controls	165		263	28.5× 9.0	1.135	0.630	2.608	
B. Effects produced by the respiratory infection, the so-called snuffles									
3	120	165	120 day	173	19.5× 4.4	0.198	0.261	0.737	Poor development
2	controls	165		180	15.5× 4.4	0.185	0.270	0.986	
5	85	130	85 day	156	19.0× 4.7	0.151	0.213		Poor development
C. Effects of raising the surrounding temperature to about 38.5°C.									
6		120		108	18.0× 7.2	0.368		0.671	Testis condition: cryptorchid
1	control	120		170	30.0× 8.5	1.120	0.539	2.097	Normal

control group in each table as contrasted with the infected and non-infected group left no doubt that the infection of 'snuffles' was the controlling factor.

The testes of the infected group, although smaller, were firm and exhibited an almost normal distribution of the various cells. There were fewer spermatids transforming into spermatozoa and fewer spermatozoa in the epididymis. The injury to the germinal epithelium was confined to the spermatidal transformation stages. In some tubules there was distinct sloughing of these cells. However, there were many perfectly normal tubules and the first impression was that the entire histological picture was normal. The interstitial cells, while normal in number, showed the same slight atypical condition observed in inanition and vitamin B deficiency (5). At autopsy the seminal vesicles of the infected group were flaccid, and the fluid was thin and clear rather than opaque and viscous as in normal vesicles. The epithelium was about half way between the normal and the castrate type. In the non-infected group there was no difference between the histology of the grafted and the control groups. The ovarian grafts were suppressed in the infected group. The largest percentage of grafts to remain vitalized was found in the immature ovarian transplant

group in which no lutein tissue was present. At autopsy, 4 of the 6 highly luteinized ovaries showed only stroma, often unorganized.

Effects of increasing the body temperature. In the respiratory infection of 'snuffles' there was a rise in body temperature, and since a slight increase in temperature has been shown to be destructive to the germinal epithelium, an increase in body temperature might also have a suppressive action on the hormone producing elements of the testis. An improvised incubator was constructed to contain 2 rat cages. The rat is somewhat poikilothermous and not very efficient in regulating its body temperature. The body temperature of young rats increases 0.5° for each 5° rise in the surrounding temperature (6), between 10° and 30° and from 30° to 35°C. the increase in body temperature is only 0.3°. However, a series of preliminary experiments showed that above 37.5°C., if the animal has not been acclimated, the body temperature usually remained about 1°C. higher than the surrounding temperature. The animals were not able to withstand temperatures above 40°C. for much more than 15 hours. Many animals died after 36 hours at 38°C. There was some indication that they made an adjustment to a gradual increase in temperature but they seemed to have no method of bringing the body temperature below that of their surroundings. Therefore, the incubator was kept at about 38.5°C. (39°C. the highest and 37.5°C. the lowest) for 15½ hours of every 24. Six animals, 100 days of age, were kept under these conditions for 15 to 21 days. The animals were definitely uncomfortable while the heat was turned on and showed a weight loss of about 26 per cent. One animal which had been used several times in testing the response of body temperature to that of the surroundings, was almost able to maintain his body weight but the body temperature always remained about 1°C. above the outside temperature. The temperature of those that became greatly emaciated more nearly approached the exact temperature of the surroundings.

At autopsy the testes of these animals were nearly the same size as those in the 'snuffles' group but they were very flaccid and weighed much less. The histological picture was that of the typical cryptorchid testis except for the interstitial elements. The maturing sperm were the first to be effected and were quickly sloughed into the lumen of the tubules. These were followed by the spermatids and the secondary spermatocytes so that at the end of 15 days only spermatogonia and primary spermatocytes remained. However, in spite of the rapid degeneration there was no evidence of clumping of the sloughed germ cells. There was no increase in the interstitial cells. The animals were killed by an accidental sudden rise in temperature and remained at this temperature for an hour or so before the tissues were preserved. The degenerative changes thus obscured the finer cytology of the interstitial cells so that it was not certain whether any changes occurred. The flaccid condition of the testis was probably due to the rapid shrinkage of the seminiferous tubules.

The seminal vesicles were not suppressed as much as in the group affected by 'snuffles' (table 1, *C*). The fluid was scanty but normal in color and consistency. The epithelium varied in height but on the average was slightly higher than in the case of the animals with 'snuffles.' The prostate also showed castrate changes in both weight and epithelial height.

Effect of diphtheria toxin on male hormone production. It was apparent that there was a great amount of toxicity in the respiratory infection, the so-called 'snuffles.' Therefore, the effects of toxicity on the production of male hormone was measured separately from any effects of increased body temperature. It was decided to use diphtheria toxin[2] as it was thought to be general in action and, since the rat is not susceptible to diphtheria, varying degrees of toxemia could be easily obtained. The doses ranged from the amount necessary to produce the first symptoms of intoxication to an almost 100 per cent lethal dose. Twenty-five experimental and 4 control males (100 days of age) were used but only those with definite intoxication and sub-lethal effects are recorded here (table 2).

There was a slight rise in body temperature, averaging 0.5°C., immediately

TABLE 2. EFFECTS OF DIPHTHERIA TOXIN UPON THE SEXUAL FUNCTIONS OF THE MALE RAT.

Rat No.	Body wt. autopsy	Diphtheria toxin injected	Autopsy after	Size of seminal vesicles	Wt. of seminal vesicles	Wt. of prostate	Wt. of testes	Condition of germinal epithelium
	gm.	cc.	days	mm.	gm.	gm.	gm.	
2382	135	3	22	25.0× 6.5	0.523	0.117	1.555	Recovery, mature sperm
2383	148	4	14	18.0× 6.0	0.285	0.225	1.234	Recovery, spermatids
2411	135	4	14	21.0× 5.5	0.270	0.130	1.208	Mitosis, a few spermatids
2414	145	4	14	16.0× 4.5	0.196	0.150	1.075	Recovery, mitosis
2422	115	3	6	22.0× 6.0	0.213	0.200	2.206	Clumping and removal of sperm
2423	118	3	10	20.0× 4.5	0.260	0.103	0.920	Only a few spermatagonia
2424	132	3	13	20.0× 6.0	0.447	0.474	1.018	Degeneration, some recovery
2425	128	3	16	24.0× 7.0	0.381	0.318	0.902	Mitotic activity of spermatocytes
2431	100	2	13	19.0× 3.5	0.162	0.140	1.273	Giant cells, some recovery
2432		2	4	16.0× 6.0	0.225		1.950	Removal of sperm
2433	90	2	8	16.0× 4.0	0.150	0.194	.855	Clumping of spermatocytes
2435	130	2	16	20.0× 5.0	0.246	0.307	1.805	Recovery, a few sperm
2390	175	control		29.0× 9.5	1.232	0.623	1.998	Normal
2415	170	control		28.0× 9.5	0.960	0.355	2.249	Normal
2416	148	control		30.0×10.0	1.117	0.365	1.950	Normal
2436	159	control		24.0×10.0	0.802	0.366	1.826	Normal

following the injection (single dose) of the larger volumes of toxin. This quickly dropped to a subnormal level and was paralleled by the weight loss of the animal. The body weight loss in severe but sub-lethal toxemia was about 25 per cent in 6 days and the body temperature dropped simultaneously from 38.2°C. to 34.8°C. Both rose rapidly during the next 4 days. The body temperature came back to normal but the recovery in body weight at this time was only about 50 per cent and was not complete at the end of the experiment (21 days). With divided doses, given several days apart, the body weight loss was about the same but was, of course, distributed over a longer period of time and was accompanied by a similar lowered body temperature.

At autopsy, the testes while somewhat smaller than those of the controls, were normal in appearance except in the most extreme cases when they were slightly less firm than normal, but they never reached the degree of flaccidity observed when the body temperature was raised. There was a shrinkage of the seminiferous tubules and a degeneration of the germinal epithelium which was similar to the changes that occur due to vitamin E deficiency. In severe toxemia the four morphological changes in germ cells described by Mason (7) were found. However, these changes occurred within a week to 10 days after

[2] The diphtheria toxin was supplied through the courtesy of Dr. John Y. Sugg of the Department of Bacteriology and Immunology, Cornell University Medical College. The author wishes to thank Dr. Sugg and Dr. James R. Dawson, Jr., also of the Department of Bacteriology and Immunology for their helpful advice in regard to the administration of the toxin.

the injection and in one case the sloughing of the spermatogonia was almost completed on the 10th day after injection (fig. 1). These testes contained only a very few residual spermatogonia and were to a great extent free from degenerating cells; the epididymis, however, contained large masses (fig. 2). By the 6th day, when the first histological study was made, the fusion and removal of the mature and maturing spermatozoa was almost complete in all tubules (fig. 3). The characteristic liquification and segregation of the chro-

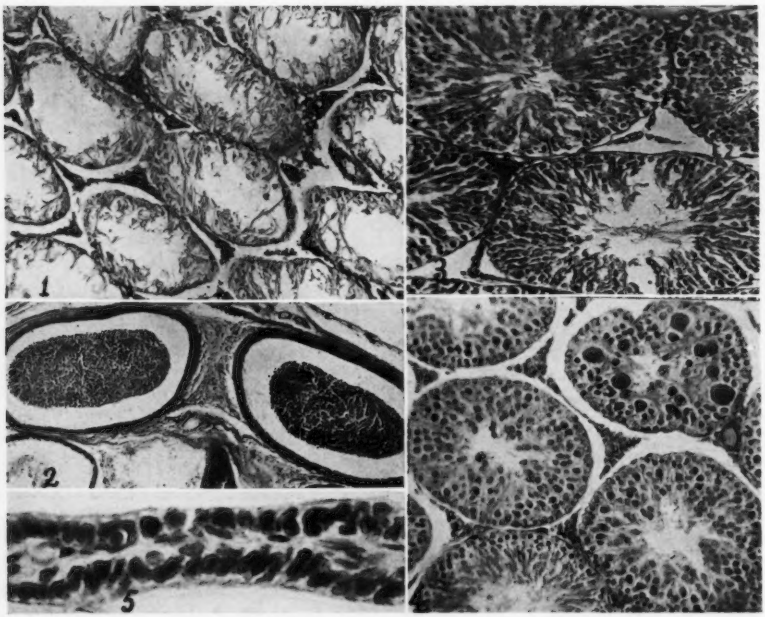

Fig. 1. Cross section of the testis of a rat 10 days after receiving 3 cc. of diphtheria toxin. Some tubules have no germ cells and some contain only a single layer of spermatogonia. At no place are they more than two cells in thickness. This is an extreme case of damage to the germinal epithelium caused by diphtheria toxin. × 80.

Fig. 2. Cross section of a portion of the epididymis, showing the mass of cellular debris that is cast off (Same animal as fig. 1). × 52.

Fig. 3. Cross section of the testis of a rat 6 days after receiving 3 cc. of diphtheria toxin. The portion of tubule at the upper right contains some apparently normal spermatids. The remainder of the tubules show various stages of the cytolysis, clumping and sloughing of the spermatids and sperm. × 130.

Fig. 4. Cross section of the testis 8 days after injection. In the lower left tubule there are some cytolysed spermatids. In the left center and lower right is seen the characteristic ring or beadlike arrangement of the chromatin of the spermatids and secondary spermatocytes. The tubule at the upper right shows the characteristic clumping of the spermatids and secondary spermatocytes to form giant cells. × 130.

Fig. 5. A longitudinal section of a portion of the epithelium of the seminal vesicle 14 days after injection with diphtheria toxin. Note the low cuboidal cells typical of the castrate condition. × 530.

matin material of the nucleus of the spermatids and the secondary spermatocytes, to form a crescent was evident in the tubules (fig. 4). These cells were already fusing into the multinucleated mass in some tubules. However, these last stages were present to a greater extent 7 to 10 days after the injection, depending upon the degree of toxemia. The actual removal of the spermatogonia and primary spermatocytes was not clearly demonstrated here, as in all

but the single case mentioned above, many spermatogonia were still present. Some of them were undergoing nuclear degeneration but it was thought that the few multinucleated masses still present were composed of secondary spermatocytes and that the spermatogonia sloughed off without much aggregation.

The interstitial cells were normal in number but the cytoplasm appeared to be shrunken and the nuclei were often rather atypical. The seminal vesicles appeared to undergo, in some cases, almost complete castration changes (depending upon the duration of the toxemia) and contained clear watery fluid. In the control the fluid was voluminous, opaque and viscous. The histological study confirmed the macroscopical findings. The epithelium of the small empty glands resembled that of the 10-day castrate (fig. 5) and the others showed varying degrees of suppression. The histological picture of the prostate agreed with that of the seminal vesicles. Both conditions were probably due to the almost immediate suppression of the male hormone. However, the appearance of the seminal fluid and the histology of the seminal vesicles and testis of the animals autopsied after 16 to 22 days and showing rapid recovery from the toxemia indicated a recovery of hormone production and spermatogenesis. The germinal epithelium and hormone-producing centers of the testis were both damaged but the extent of damage of one was not necessarily correlated with the other, as the seminal vesicle of the animal with only a few spermatogonia did not show as great a depression as several with much less germinal epithelial damage.

Male hormone production during a short period of inanition. There was paralysis and loss of appetite due to the toxicity in the animals injected with diphtheria toxin. Further, when the body temperature and the body weight were plotted against time the resemblance to the curves for total inanition was readily seen. Inanition has been definitely shown to suppress male hormone production (5) and therefore 6 males, 100 days of age, were starved for 6 days to produce a weight loss similar to that in the diphtheria toxin group. The body temperature did not fall below 36.0°C. The animals were autopsied at the same time as those in the toxin group. There was no effect upon the germinal epithelium but there was some suppression of male hormone after 10 days, especially in those animals showing the greatest emaciation. However, recovery was rapid and later autopsies demonstrated normal sexual accessory organs. Therefore, the effects observed in the diphtheria toxin group were apparently not due to inanition.

DISCUSSION

These experiments demonstrate that there is no antagonism between the grafted ovaries and the gonads of the host. However, the condition of the ovary to be transplanted is very important in the vitalization of the graft, especially in the normal male in which the follicle-stimulating hormone is in equilibrium with the male hormone produced by the testis. In the castrate, the increased follicle-stimulating hormone counteracts the influence of the degenerating lutein tissue so that the follicular development is maintained.

Ovarian transplants between different strains of mice are not successful if the strains are unrelated (8) and there is a definite correlation with the relationship of the strains. Therefore it is remarkable that the mouse-ovary transplant persisted as long as it did in the eye of the rat.[3] However, Crisler (9) reported the persistence of mouse testicular tissue in the scrotum of the rat after 5 or 6 months. It is possible that the fluid of an anterior chamber of the eye acts as a tissue culture media for the graft and vitalized inter-strain grafts may be obtained by this method.

The indication that the rat can make some adjustment to an increase in body temperature is in agreement with the work of Prizibraum (10) but in these short experiments they were unable to lower their body temperature below that of the surroundings. That the rise in body temperature affects primarily the germinal epithelium as does heat applied locally to the scrotum (11) indicates that other factors are involved in the suppression of the male hormone in the case of the respiratory infection. However, there was some suppression of the male hormone, which was not observed in the cryptorchid except after long time periods. It might seem that inanition is the cause of the hormone suppression, especially since the interesting observation of Moore and Samuels (5) that inanition can produce complete castrate changes in the accessory sexual glands without much interference with the germinal epithelium. This does not rule out the effect of bacterial toxins which, from these investigations, is shown to exert the greatest influence upon the suppression of the male hormone and also in some cases the greatest destruction of the germinal epithelium. Nevertheless, these two factors are not necessarily correlated and the longer action of the toxin as produced by the organism during the infection might cause a great suppression of one, the hormone production, without serious damage to the other. Also, under these conditions the germinal epithelium may show a more rapid recovery.

The diphtheria toxin must act directly upon the germinal epithelium and not through a suppression of the hypophysis because 6 to 10 days of toxemia can produce a greater destruction of the germinal epithelium than a month's absence of the pituitary (12). The spermatogonia are present for some time after hypophysectomy, but may be almost completely destroyed 10 days after the injection of diphtheria toxin. In the degenerative changes during the sloughing of the germinal elements, the testes resemble those seen after vitamin E deficiency but there can be no direct connection as these changes occur only after 50 to 100 days on the vitamin E free diet and require 35 to 50 days for completion (7). Also, there is no suppression of the male hormone except after very prolonged absence of the vitamin (13). The suppression of the hormone-producing centers by the toxin may be due to a secondary action of suppression of the pituitary gland, since the changes in the secondary sexual accessories resemble those following hypophysectomy. However, the action is

[3] Ovarian grafts seldom remain vitalized under the skin of the ear of normal male or female mice (related strains) but always in castrates. They regress and lie dormant for many months in the normal animals, and show no physiological effect upon the mammary gland of the male host. However, after castration of the host, these dormant ovarian grafts grow rapidly and reach a stage of development equal to that occurring when the castration was performed before transplantation. (Personal communication from Dr. R. T. Hill, Department of Anatomy, Yale University Medical School).

more immediate than is the case in inanition, which has been definitely shown to act through the suppression of the pituitary (5). This can only be shown by administration of the gonadotropic hormones.

SUMMARY AND CONCLUSIONS

Mouse ovaries do not remain vitalized in the anterior chamber of the eye of the rat, although ovarian stroma may be recognized 35 to 40 days after transplantation. Excessive amounts of lutein tissue are unfavorable for successful vitalization of an ovarian graft in the normal male rat. There is no antagonism in the rat between the grafted ovary and the gonads of the host. Suppression of the graft and the male hormone in these experiments is due to factors introduced by a respiratory infection, the so-called 'snuffles.' The effects of these various factors are analyzed separately.

A rise in body temperature to 39° or 40°C. for 15½ hours of the 24, obtained by raising the surrounding temperature, causes typical cryptorchid changes in the testes and some suppression of male hormone.

Toxemia, produced by injection of sublethal doses of diphtheria toxin, produces degenerative changes in the germinal epithelium which resemble effects of vitamin E deficiency except that almost complete removal of the germinal epithelium may occur within 10 days from the time of injection. The male hormone may be completely suppressed for a time as indicated by seminal vesicle histology. There is recovery of both spermatogenesis and hormone production. The toxin probably acts directly upon the germinal epithelium. However, the effect upon the hormone producing centers may be due to a suppression of the hypophysis.

Inanition over the same period as the weight loss in the toxemia experiment has no effect upon the germinal epithelium and causes only a slight suppression of the male hormone.

Toxemia is the main factor in the suppression of the male hormone due to 'snuffles' infection, although fever and partial inanition may be contributing factors.

REFERENCES

1. Allen, E.: Sex and Internal Secretions, Chapters IV and V. The Williams and Wilkins Co., Baltimore, 1932.
2. Moore, C. R., and D. Price: Am. J. Anat. **50:** 13. 1932.
3. Pfeiffer, C. A.: Anat. Rec. **65:** 213. 1936.
4. Pfeiffer, C. A.: Am. J. Anat. **58:** 195. 1936.
5. Moore, C. R., and L. T. Samuels: Am. J. Physiol. **96:** 278. 1931.
6. Donaldson, H. H.: The Rat. The Wistar Institute, Philadelphia, 1924.
7. Mason, K. E.: Am. J. Anat. **52:** 153. 1933.
8. Strong, L. C.: J. Hered. **27:** 218. 1936.
9. Crisler, G.: Am. J. Physiol. **90:** 623. 1929.
10. Prizibraum: Cited by Donaldson (6).
11. Moore, C. R.: Am. J. Anat. **34:** 337. 1924.
12. Smith, P. E.: Am. J. Anat. **45:** 205. 1930.
13. Kudrjaschov, B. A.: Endokrinologie **7:** 91. 1930.

PRODUCTION OF ESTROGENIC HORMONE BY A TRANSPLANTABLE OVARIAN CARCINOMA

LEONELL C. STRONG,[1] W. U. GARDNER AND R. T. HILL[2]
Department of Anatomy, Yale University School of Medicine
NEW HAVEN, CONNECTICUT

Physiological activity of glandular neoplasias has been frequently described. Tumors involving the endocrine glands are of particular interest for profound changes may be brought about in the individual by the hormones secreted by such neoplasms. That these tumors secrete hormones is shown by alleviation of the aggravated conditions after surgical removal of the tumors.

Among the tumors of the endocrine glands that are of interest are those involving the ovary. Impetus toward the study of the problem of ovarian tumors secreting hormones has been accelerated by the series of papers by Meyer (1-5) since 1930. At the present time in mankind, according to Meigs (6) 6 ovarian tumors which may secrete hormones have been identified: *1)* ovariotestes; *2)* testicular tubular adenoma of Pick; *3)* arrhenoblastoma; *4)* hypernephroma of adrenal origin; *5)* disgerminoma or seminoma; and *6)* granulosa cell tumor. Of these 6 types only the granulosa cell tumor is known to secrete estrogenic hormone.

The origin of the cells of the granulosal cell tumor is in very great dispute, but the primitive cells that make up the original cells of the Graafian follicle are probably responsible. That the granulosal cells of the adult follicle are responsible is somewhat questionable. Transplantation has been of value in the study of many types of physiological functions. Grafted pituitaries may function normally (7) and transplanted ovaries may continue to secrete ovarian follicular hormone (8).

In this paper evidence is submitted that grafted carcinoma of the ovary secretes estrogenic hormone. Mouse S 108,120 of the CBA strain was put with her brother at 57 days of age, and separated 388 days later. During this interval 6 litters of young were born. At the time the male was removed from the cage, the female was placed in reserve on a standard Purina-fox chow diet. Forty-five days after the male had been removed, and when the female was 490 days of age, an abdominal enlargement was observed and she was killed. The peritoneal cavity was filled with a pale reddish fluid. The right ovary was replaced by a solid tumor mass measuring 3.0 $\times$ 2.2 $\times$ 2.2 cm. (fig. 1). The tumor was firm and glistening white. Around one margin was a narrow mass of dark hemorrhagic tissue. The uterus was distended, similar to the estrous condition. All other viscera appeared to be normal.

[1] This experiment has been made possible by grants from the Josiah Macy, Jr., Foundation and the International Cancer Research Foundation.
[2] It has also been aided by grants from the Fluid Research Funds of Yale University, School of Medicine.

The tumor was diagnosed as an adenocarcinoma of the ovary arising presumably from follicle tissue (fig. 2). For the most part, the tumor was composed of compact masses of small polygonal cells (fig. 3). In many areas these dense masses were broken up into small alveolar structures. The cells in the compact masses resembled follicular cells with deeply staining nuclei and

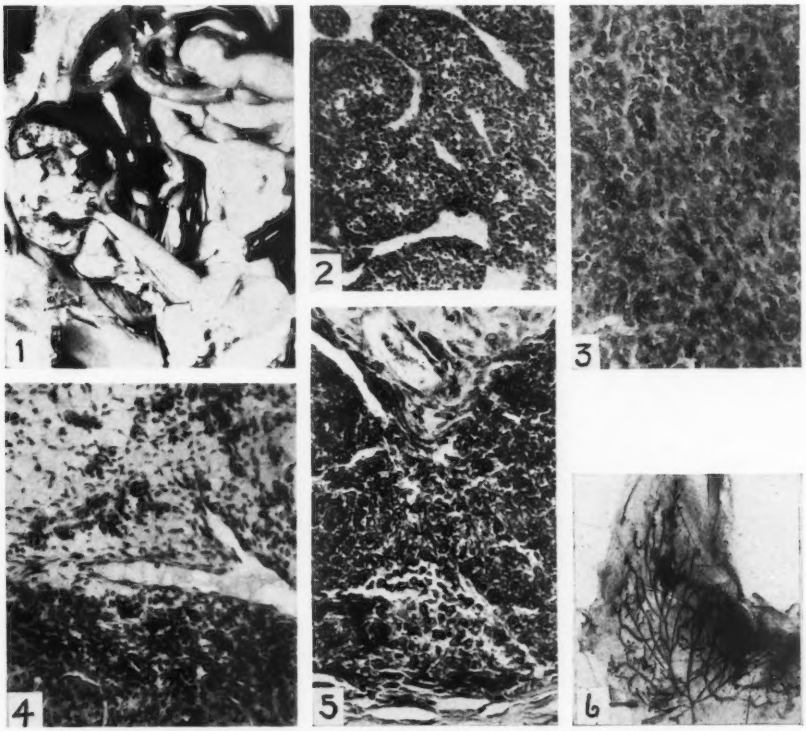

Fig. 1. Photograph of the viscera of mouse S 108,120, showing the carcinoma of the right ovary (left central). The uterus (lower central) is greatly distended. ×1.

Fig. 2. Photomicrograph of the carcinoma of the ovary from mouse S 108,120. The tumor tissue is arranged in a papillary manner presumably indicating its origin from a graafian follicle. ×71.

Fig. 3. Photomicrograph of the same carcinoma of the ovary showing the cell structure in one of the compact masses. ×113.

Fig. 4. Photomicrograph of the same carcinoma of the ovary showing an area of compact tumor tissue and an area of fibrous connective tissue. ×113.

Fig. 5. Photomicrograph of the grafted tumor in mouse TV 13. The tumor tissue is seen between the capsule of the graft (below) and a spicule of bone (above). ×113.

Fig. 6. Photograph of the mammary gland from male mouse TV 4, showing the branching arrangement of the ducts as a result of stimulation by estrogenic hormone. ×5. The control rudimentary gland of the untreated male would be equivalent to a single terminal branch of the experimental gland.

scant cytoplasm. Mitotic figures were frequent. Scattered throughout the tumor were small cystic areas resembling follicular spaces containing albuminous material and desquamated tumor cells without any evidence of blood. In other places the arrangement of cells assumed a papillary character (fig. 2). In such places the branching cords of cells were supported by delicate cores of connective tissue frequently accompanied by small blood vessels. The tumor was traversed by irregular bands of dense fibrous connective tissue (fig. 4) in

which small bony spicules were found. In other areas the connective tissue was loosely arranged and edematous. In several places, the fibrous tissue assumed osteoid characteristics. Fatty tissue was observed frequently in the stroma (fig. 4). There was no evidence of inflammation or hemorrhage. Several small areas of necrosis were scattered throughout the tumor. No ova could be found in the sections examined. No lutein-like cells were present.

A small piece of the original tumor was grafted into each of 3 female mice and into 1 male. The tumor tissue grew in the male and in 2 females. From this growing transplanted tissue a total of 99 mice have so far been inoculated (7 transplant generations). Successful growth of the tumor has resulted in 32 individuals (fig. 5). The growth rates of the grafts in 3 female mice are shown in figure 7.

The evidence of the endocrine activity of this tumor in the production of

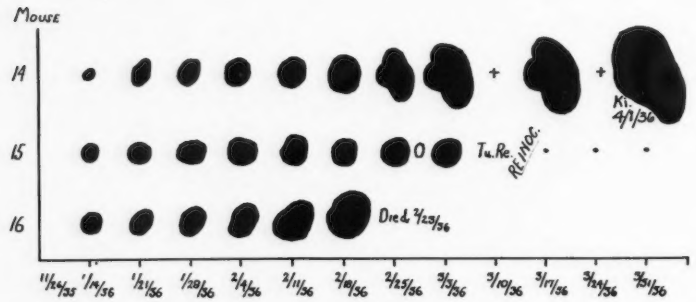

Fig. 7. Showing rate of growth of grafted ovarian carcinomata in 3 female mice, TV14, TV15 and TV16. No. of weeks after inoculation is indicated along the base line. *O* = ovariectomy; *Tu. Re.* = tumor removed; *Ki.* = killed.

estrogenic hormone was determined by *1)* following the vaginal smears in 3 female mice and *2)* observing the development of the mammary glands of 4 male mice bearing grafts of the tumor.

EXPERIMENTAL DATA

Vaginal smears. Implants from the original tumor were made into 3 females, TV 14, 15 and 16. Daily vaginal smears were started 51 days later. One mouse (TV 14) showed continuous estrous smears for 80 days, at which time the tumor was 3.5 × 2.5 × 2.0 cm. in size. The animal had become emaciated and was killed. The second mouse (TV 15) showed continuous vaginal cornification for 41 days. The ovaries were then removed and estrous smears continued for 13 days. At that time, the large tumor was removed, and the mouse reinoculated. Four days after the removal of the tumor the vaginal smears returned to the diestrous condition for 52 days. At this time the reinoculated tumor could barely be palpated between the finger tips. Mouse TV 16 gave estrous smears for 16 days at which time she was ovariectomized. Vaginal cornification continued for 6 days after ovariotomy and then the animal died.

Mammary glands. The mammary gland rudiments of 4 male mice growing

the tumor from the second to the fourth transplant generations were examined. The animals were killed at 49, 61, 105 and 108 days respectively, subsequent to inoculation at which time all the tumors were over 1 cm. in diameter. The mammary glands of all of the males had grown into extensive duct systems similar to those observed in male mice bearing ovarian grafts (8) or receiving optimal amounts of estrogenic hormones for comparable periods of time (9, 10) (fig. 6).

GENERAL DISCUSSION

Haaland, (1911) failed to obtain viable grafts of an adenocarcinoma of the ovary inoculated into 120 mice. The success of our experiments in obtaining progressive growth of the ovarian carcinoma in approximately 35 per cent of all individuals inoculated may be ascribed to the high degree of inbreeding to which these mice have been subjected over a period of 17 years.

The estrogenic activity of ovarian tumors has been observed in the case of granulosal cell tumors in women. Evidence of estrogenic activity has also been seen in one mouse with bilateral granulosal cell tumors (11). As far as we are aware the estrogenic activity of other types of malignant ovarian epithelial tumors has not been demonstrated. The infrequent occurrence of ovarian tumors in experimental animals has increased the difficulties involved in studying their physiological activity.

The present investigation has demonstrated the estrogenic activity of a second variety of ovarian tumor, (differing in histological details) namely, carcinoma of the ovarian follicle. Though extensive growth of the mammary ducts had been induced, the type of growth was similar to that of mice receiving small amounts of estrogenic hormone or of male mice bearing ovarian grafts. Considering the large amount of tissue present in those male mice bearing the grafts of the ovarian carcinoma the production of hormone was relatively slight. The pubes of male mice receiving large amounts of estrogenic hormone become partially resorbed and separated by interpubic ligaments (12, 13). This loosening of the symphsis frequently occurred in male mice bearing ovarian grafts. As the pubic symphyses of the mice bearing the grafts of the ovarian carcinoma were firm or only very slightly loosened, it was further indicated that the hormone production was comparatively low.

It is obvious that the two varieties of ovarian tumors in mice so far described and investigated may be fundamentally derived from follicular tissue. Neither type, however, has exactly the same structure as do the granulosal cell tumors in humans. The importance of the present contribution lies not in the specific origin of the cell type but primarily in the fact that an ovarian tumor continues to remain physiologically active in the production of estrogenic hormone, during the process of transplantation.

Opportunities for further investigations on the histological origin of ovarian tumors may shed light on the problem of cell type responsible for the production of estrogenic hormone.

SUMMARY

The evidence presented demonstrates that transplanted ovarian carcinoma

(granulosal cell) secretes appreciable amounts of estrogen. Criteria of hormone secretion of the transplanted tumors were: *a)*, long continued estrous smears which, after removal of the tumors, reverted to the diestrous type; and *b)*, growth of the rudimentary mammary glands of males as transplanted tumors grew. There was not enough hormone secreted to affect the pelves.

REFERENCES

1. Meyer, R.: Verhandl. d. deutsch. path. Gesellsch. **25:** 328. 1930.
2. Meyer, R.: Am. J. Obst. & Gynec. **22:** 697. 1931.
3. Meyer, R.: Arch. f. Gynäk. **145:** 2. 1931.
4. Meyer, R.: Klin. Wchnschr. **9:** 2237. 1930.
5. Meyer, R.: Zentralbl. f. Gynäk. **54:** 2374. 1930.
6. Meigs, J. V.: Ann. Surg. **102:** 834. 1935.
7. Hill, R. T., and W. U. Gardner: Proc. Soc. Exper. Biol. & Med. **34:** 78. 1936.
8. Tamura, Y.: Proc. Roy. Soc. Edinburgh **47:** 148. 1926-27.
9. Gardner, W. U.: Endocrinology **19:** 656; 667. 1935.
10. Gardner, W. U., A. W. Diddle, E. Allen, and L. C. Strong: Anat. Rec. **60:** 457. 1934.
11. Gardner, W. U., L. C. Strong, and G. M. Smith: Am. J. Cancer **26:** 541. 1936.
12. Gardner, W. U.: Proc. Soc. Exper. Biol. & Med. **33:** 104. 1935.
13. Burrows, H.: J. Physiol. **85:** 159. 1935.

THE EFFECTS OF THYROIDECTOMY, CASTRATION, ANTERIOR LOBE ADMINISTRATION AND PREGNANCY UPON EXPERIMENTAL DIABETES INSIPIDUS IN THE CAT

W. R. INGRAM AND CHARLES FISHER
From the Department of Anatomy, State University of Iowa
IOWA CITY, IOWA
and the Institute of Neurology, Northwestern University
CHICAGO, ILLINOIS

In a series of papers dealing with the experimental production of polyuria in cats and monkeys (1, 2, 3) evidence has been presented to show that permanent polyuria as produced in such forms is associated with lesions interrupting the nervous pathway extending from the nucleus supraopticus to the posterior hypophysis. In these cases there is atrophy of the supraoptic nuclei and of the pars nervosa of the hypophysis, and such atrophic glands have been found to be deficient in content of antidiuretic, pressor and oxytocic principles (4). It has been further shown that polyuria may occur in cats following direct removal of the posterior lobe including the stalk, providing functional anterior lobe tissue is left (5). Cats with diabetes insipidus, or even a latent tendency that way due to partial interference with the supraoptico-hypophyseal system, are unusually sensitive to the diuretic effects of thyroid and salt feeding, but thyroidectomy, while diminishing the intensity of the polyuria, has not in our experience completely eliminated the excessive water exchange (6). The present communication deals with further experiments on the effect of thyroidectomy upon cats with diabetes insipidus, and with other observations on the influence of castration, administration of a crude anterior lobe extract and pregnancy.

METHODS

The operative techniques involved in the production of diabetes insipidus or in the direct approach to the hypophysis in cats have already been described (1, 5). Removal of the thyroids was performed in the usual manner, an effort being made to leave at least one of the external parathyroids in situ. At autopsy thorough search was made for accessory thyroid tissue, any doubtful bits being sectioned and examined microscopically.

The crude anterior lobe preparation used was a saline suspension of fresh beef glands prepared according to Schockaert (7), and injected intraperitoneally twice daily in doses equivalent to 1.5 to 3.0 gm. of fresh tissue per day. The animals were kept under uniformly favorable conditions. The diet was kept as constant as possible and consisted of milk and a mixture of ground Purina dog chow and fresh beef kidney. The water exchange and specific gravity of the urine were carefully noted each day.

OBSERVATIONS

Effect of thyroidectomy. Three cats thyroidectomized in a previous series of experiments (6) showed a reduction of the water exchange by from a fourth to a half, but without completely abolishing the polyuria. However, Mahoney and Sheehan (8) found that removal of the thyroids in dogs with diabetes insipidus produced by occlusion of the pituitary stalk entirely eliminated the polyuria and polydipsa. Therefore 6 more polyuric cats of our group were thyroidectomized. Two of these developed tetany and respiratory tract disease and were discarded. The others were observed from 6 to 277 days after removal of the thyroids.

The general results are summarized in table 1. Some comment may aid in the interpretation of these data. In cat 1 the first 23 days of the permanent

TABLE 1. THE EFFECT OF THYROIDECTOMY ON POLYURIA.

Cat	Average Daily Water Exchange Before Thyroidectomy		Days	Average Daily Water Exchange After Thyroidectomy		Days	Remarks
	Urine cc.	Fluid cc.		Urine cc.	Fluid cc.		
1	122(1.018)	130	23				
	225(1.015)	337	26	243(1.017)	311	77	
				—	—	33	Out of cage
				208(1.026)	261	40	
				Thyroid feeding,		6	
				382(1.015)	425	98	
2	229(1.013)	382	18	275(1.015)	312	25	
3	358(1.014)	375	15	221(1.025)	221	6	Died
4	412(1.012)	579	9	206(1.019)	316	57	

polyuric phase was complicated by illness and the average daily fluid exchange was relatively low. During the next 26 days, however, a fairly good polyuria was manifest and at the end of this period the thyroids were removed. The average figures for the next 77-day period disclose no significant change. Some gradual decrease in urine output did occur later. For about a month the cat was out of the metabolism cage, but for the 40 days following some reduction in fluid exchange was evident. The animal developed a stupid irritable attitude and expression, slept a great deal and showed thickening of the eyelids and some loss of hair. Dessicated thyroid in 1-gram doses was then fed for 6 days. This caused a decided increase in urine output and an improvement in the general status of the cat. It is of interest that once the marked polyuria was resumed it persisted long after the effects of the thyroid feeding must have passed off; indeed, the water exchange reached its peak during the last 12 days of survival.

The remaining cases require little comment. It is evident that thyroidectomy as performed in 4 cats with permanent polyuria failed to abolish the polyuria but effected some reduction in the water exchange in each case, the greatest reduction amounting to about 50 per cent.

Effect of castration. The experience of Schlotthauer (9), who reports that castration alleviated diabetes insipidus of unknown etiology in a 1-year-old bull, has led us to study the effects of castration in 4 cats with experimental diabetes insipidus of varying intensity. One of these, cat 7, was an adult female, one, cat 8, was a large adult male, and two, cats 5 and 6, were immature males weighing 2.07 and 2.5 kg. respectively.

Table 2 summarizes the essential data accruing from these experiments, and shows clearly that castration does not bring about any significant decrease in fluid exchange in animals of the type dealt with. In fact, in the case of the female, cat 7, a decided increase in the output of urine followed ovariectomy.

Effect of anterior lobe injection. Diuresis in animals as a result of treatment with anterior lobe extracts has been reported by a number of investigators (10, 11, 12). There seems to be some doubt as to which of the numerous

TABLE 2. THE EFFECT OF CASTRATION ON POLYURIA.

Cat	Average Daily Water Exchange Before Castration		Days	Average Daily Water Exchange After Castration		Days
	Urine cc.	Fluid cc.		Urine cc.	Fluid cc.	
5	651(1.008)	817	81	662(1.010)	787	50
6	369(1.012) 341(1.017)	451 471	100 27	351(1.019)	414	57
7	205(1.025)	267	24	324(1.010)	345	54
8	255(1.021)	352	16	241(1.021)	310	43

anterior lobe hormones are concerned. According to Barnes and his co-workers and Biasotti, it is chiefly the thyrotropic principle, since their extracts had no effect when administered to thyroidectomized dogs. Gaebler (13), on the other hand, observed diuresis in thyroidless dogs after antuitrin-G injections. In our hands, a crude anterior pituitary suspension, while effective in elevating the blood sugar in many instances, had practically no effect on the water exchange in normal cats. For instance, in a group of 4 normal cats receiving the equivalent of 1.5 gm. fresh gland daily for 7 days, the following water exchange averages were noted during and after the period of injection:

Cat X1—urine 140 cc. (specific gravity 1.033), fluid intake 100 cc.; 7 days.
Cat X2—urine 133 cc. (1.031), fluid intake 100 cc.; 7 days.
Cat X3—urine 131 cc. (1.037), fluid intake 107 cc.; 12 days.
Cat X4—urine 108 cc. (1.033), fluid intake 100 cc.; 17 days.
Cat Z1—(Receiving no extract) urine 123 cc. (1.029), fluid intake 100 cc.; 14 days.

These figures are within the normal range for cats. The uniformity in fluid intake is due to the fact that the daily milk ration was 100 cc. and was almost invariably consumed by healthy cats, with rarely any additional water. Numerous other normal cats given similar or larger doses of the suspension have likewise failed to demonstrate significant increases in water exchange.

Three groups of operated animals have also been subjected to this treat-

ment. The first group, consisting of 5 cats with lesions involving the walls of the third ventricle dorsal to the chiasm and extending into the preoptic area, but without polyuria, showed no significant diuretic response to the extract. These are not included in table 3.

The second group also consisted of 5 animals, cats 6, 7, 9, 10 and 15 (table 3). Cats 6 and 15 had marked permanent polyurias. Cat 7 had a mild and variable polyuria. Cats 9 and 10 were somewhat atypical cases. Cat 9 had had a rather marked polyuria for 26 days as indicated in table 3, but this became greatly reduced during a prolonged illness. Cat 10, although it had shown a sudden increase in urine output for 2 days at about the usual time for the onset of the permanent phase of the polyuria, had shown nothing further. The results in this group are shown in table 3, and are rather equivocal. Cats 6 and 15, which had rather marked polyurias to start with, showed no diuretic response. Cat 9 showed a decided increase above the level for the period immediately preceding injection, but hardly an increase over the original level. Cat 7 manifested a not very striking increase after 10 days of injection, but after 16 days of observation was removed from the metabolism cage for 2 weeks. When replaced, it was evident that the increase had persisted and become more marked, as shown by the average for 24 days. Cat 10 responded with a striking increase, reaching 850 cc. of urine on the last day of injection. The urine output then returned to normal but in the 32 days following, gradually increased until a level of 228 cc. was reached. The anterior lobe suspension was again administered over a 5-day period, and again an increase was recorded, the urine output for the 19 subsequent days averaging 383 cc.

The third series consisted of 4 cats, in 3 of which total hypophysectomy had been attempted (cats 12, 13 and 14) and in one of which (cat 11) the infundibular stalk had been removed. In 2 of the hypophysectomized cats, 12 and 13, the anterior lobe was subsequently found to have been all removed except for a few degenerate cells. As will be seen from table 3, these animals showed no polyuria before anterior lobe administration, which was carried out about one month after operation. Following this treatment, fairly mild polyurias developed rather gradually, and strangely enough, persisted with some variation long after injection of anterior lobe had ceased. In the other so-called hyphysectomized cat, 14, a remnant of anterior lobe was later found to have been left in the sella. This animal developed a fairly marked polyuria following the operation, but except for some indication of increased fluid intake at the time the injections were given, no positive effects were elicited by giving anterior lobe suspension. Cat 11, from which the infundibular stalk was largely removed, showed a mild transient polyuria during the first 2 post-operative days. There was again a decided increase in the fluid intake on the 8th day; the urine output, however, did not increase until the 24th day, which was the fourth day after anterior lobe treatment had begun. The average figures for the next 100 days shows a decidedly excessive water exchange. There is some question whether this prolonged increase was due to the effects of the anterior lobe injections, or whether it was merely the normal onset of the

polyuria, delayed in this case somewhat beyond the normal latent period.

To summarize, 2 cats with marked polyuria resulting from hypothalamic lesions yielded no increase in water exchange in response to anterior lobe treatment, 1 cat gave a rather doubtful positive response, and 2 atypical animals gave positive results. Two hypophysectomized cats were found to respond to anterior lobe treatment with increased urine output. The higher rate of exchange persisted long after cessation of the injections. One partially hypophysectomized cat which showed a polyuria before anterior lobe administration, failed to respond. In another cat, from which the infundibular stalk had been

TABLE 3.—THE EFFECT OF ANTERIOR LOBE INJECTION ON POLYURIA.

Cat	Average Daily Water Exchange Before Injection		Days	Days Injected	Average Daily Water Exchange After Injection		Days
	Urine cc.	Fluid cc.			Urine cc.	Fluid cc.	
9	244(1.013)	232	26				
	162(1.022)	159	115[1]	7	275 (1.016)	237	41
10	Normal except for increase on 15th and 16th days. Second injection period		30	5	310(1.015)	335	1
					850(1.007)	800	1
					—	—	32[2]
				5	383(1.011)	396	19
11	100(1.031)	210	23	7	313(1.019)	451	100
7	127(1.027)	154	57	10	158(1.027)	216	16
					—	—	14[3]
					205(1.025)	267	24
12	97(1.023)	102	18	9	197(1.021)	147	45
13	94(1.021)	82	21	9	206(1.017)	194	43
14	252(1.012)	225	18	9	242(1.016)	214	45
6	369(1.012)	451	100	6	341(1.017)	471	27
15	244(1.017)	321	26	12	234(1.014)	298	22

[1] Including period of illness.
[2] Returned to normal, but gradually increased in 32 days to 228 (1.021).
[3] Not recorded for 14 days.

removed, polyuria developed after anterior lobe treatment was begun on the 20th post-operative day.

Effect of pregnancy. Duvoir, Pollet and Cachin (14) have reported a case of diabetes insipidus in a woman who during the course of the disease passed through three pregnancies. During the gestation period the urine output began to decline at about the fourth month and became normal in the weeks preceding delivery, after which the water exchange rose to its previous level. The present authors have naturally desired to study the influence of pregnancy on cats with diabetes insipidus. However, although many polyuric cats have been under close observation for long periods of time and during the breeding seasons, none has been seen to show signs of estrus, while normal cats, or certain animals with other types of hypothalamic lesions, which were kept

under similar conditions have bred and become pregnant. In an attempt to secure material for this study, 3 normal females were bred to active males and were observed to have copulated several times. A week to 10 days later they were operated for the production of diabetes insipidus. Polyuria developed in 2 of these animals and for a time it was thought that they were pregnant. As time went on, however, they failed to gain in weight or size, although no signs of abortion were noted, and when they were killed long after the usual term, no evidence of recent pregnancy was found. The same was true of the third cat, which did not show a polyuria. It seems strange that none of these cats should have conceived, but the authors shall have no explanation to offer until further work has been done along this line. In spite of this failure we are fortunate in being able to present some data in regard to the effect of pregnancy which were secured more or less inadvertently. Although necessarily scanty, they are of some interest.

Cat D38, operated March 15, developed a permanent polyuria which began on the 14th day (March 29). A marked polyuria persisted until April 8, when it diminished somewhat at the onset of an attack of 'snuffles.' The cat became quite ill and on April 12 to 15, normal saline solution was administered intraperitoneally. The animal died April 15, and at autopsy was found to be pregnant—evidently about half-term. The uterus had been damaged by the injection needle, but no abortion had occurred.

Cat D48, operated May 15, failed to show a transient polyuria, but developed a permanent polyuria after about 23 days. The polyuria being marked, removal of the hypophysis was attempted June 20. Recovery was poor and wound infection set in. On June 25 there was evidence of abortion. The cat was dead on June 26. One embryo was found in the uterus.

Cat D61, operated September 5, showed a tremendous transient polyuria lasting for 5 days, and then for 12 days the urine volume was quite low. On the 19th post-operative day the water intake and urine volume increased, the specific gravity of the latter falling. A mild, variable polyuria characterized chiefly by low specific gravity of the urine lasted until October 7. At this time the cat was found with one practically full-term fetus partly delivered. When it was obvious that no progress was being made, the fetus was removed. The cat died during that night.

From these 3 cases it is apparent that polyuria may occur in pregnant animals, in spite of the fact that in 2 of them pregnancy was more or less accidentally terminated by death. The other, although presumably at term, was unable to survive attempted parturition. The fourth instance is the most interesting and furnishes the most complete data.

This cat, D121, was operated January 6, and showed a transient polyuria on the 2nd and 3rd days. The permanent phase of the polyuria appeared after a latent period of 15 days and developed to an almost unheard of level, at times reaching 900 cc. in 24 hours. Increase in size of the abdomen was noticed early in February, and on March 2 an x-ray plate disclosed the presence of several fetuses. The animal must have been in early pregnancy when operated, as there had been no heat phenomena nor opportunity for copulation

since that time. The tremendous polyuria continued unabated until March 5, when the urine output dropped to about 400 cc. and continued at about this level for the remainder of the survival period. On March 6 the cat's appetite failed. On the morning of March 7 one well-formed, active kitten had been born, but the placenta had not been delivered and was not delivered until 2 P.M. The progress of labor was unusually slow and nothing more occurred that day. By the middle of the next morning a total of 4 kittens had been born, 2 of which were dead. The cord was still uncut on one of the live ones. No after-births were found. The live kittens were dirty but seemed vigorous. The mother was weak. Her nipples were well developed but the mammae were not large and no milk could be expressed. There was a fairly large urine volume—over 400 cc. The cat was found dead on the morning of March 9. One kitten was still alive. At autopsy 2 unborn kittens were found in the upper ends of the uterine horns. These were straightened to full length and firmly compressed. The post-operative period up to the beginning of labor was 60 days. The usual gestation period in normal cats is about 63 days.

It is clear that in the case of this cat pregnancy had no influence upon the course of the polyuria—unless it were to increase its intensity—until 2 days before delivery, at which time the urine output dropped to about 400 cc., which ordinarily would still be considered marked polyuria. This level was maintained during the more than 2 days of abnormally prolonged labor.

These rather limited data indicate that animals operated early in pregnancy may develop diabetes insipidus which lasts until term, at least. The course of the polyuria beyond this point has not been determined, since none of our animals which went into labor at or near term successfully completed delivery and recovered, nor did any cat with polyuria survive abortion so far as our records show. We have no information to offer as to what occurs in cats operated late in pregnancy—our attempts having usually ended in prompt abortion or death. One animal passed through a normal, rapid delivery of full-term kittens on the second post-operative day, but in this case the lesions were not placed so as to produce polyuria.

COMMENT

The results of our attempts in 7 cases to remove the thyroids from cats with diabetes insipidus have in some degree failed to confirm the findings of Mahoney and Sheehan in dogs, for while the water exchange was reduced, the polyuria still persisted. It should be said that it is not clear that the polyurias produced by Mahoney and Sheehan were permanent—it may be that they disappeared for reasons other than thyroidectomy. One must consider, however, that there is a possibility of overlooking accessory thyroid tissue (Marine, 15). Our results should perhaps be accepted with some reservation on this account, but it might also be pointed out that such criticism is even more applicable to experiments with dogs. Specific data as to the occurrence of accessory tissue in the cat are difficult to find, although it has been carefully studied in the dog (13, 16). Nicholas and Swingle (17) make no mention of thyroid accessories in the cat. Davis (18), although he frequently found accessories in

the periaortic and pericardial fat bodies of dogs, was unable to do so in 12 cats examined. Careful macroscopic search in our own cases has not disclosed accessory thyroids. Unfortunately, we were unable to secure basal metabolic findings in our thyroidectomized animals. The general appearance and behavior of the long-surviving cats, however, was indicative of thyroid deficiency, and thyroid feeding brought about considerable improvement in 3 instances in which it was tried.

As to the effect of castration on excessive water exchange, the results are clear cut and call for little further discussion. We can offer no rational explanation for Schlotthauer's findings, but it is clear that in both immature and adult cats such procedures have no influence in depressing the water exchange. Indeed, on theoretical grounds, the reported fact that administration of gonadal hormones may suppress polyuria (vide infra) might lead one to expect an increased urine output after gonadectomy.

The findings in regard to anterior lobe administration leave much to be desired, both as to consistency of results and their explanation. However, although we were unable to produce polyuria in normal cats by injection of the crude suspension used, positive results were obtained in 5 of 9 operated cats. One other might also be considered positive, although less clear cut. Generally speaking, one is tempted to remark that in animals with a latent tendency to polyuria, such treatment was effective, as in experiments on thyroid feeding, but the argument is rather spoiled by the fact that in 3 cats already displaying more or less marked polyuria anterior lobe administration had no effect. No other explanation, though, seems to be forthcoming, and perhaps it may be applied to cases in which a pre-existing polyuria has been depressed by illness or some other factor, or to cases in which the onset of the polyuria has been for some reason delayed. According to the von Hann (19) theory, the occurrence of polyuria depends not only upon a lack of anti-diuretic principle, but upon the presence of functional anterior lobe tissue. Subnormal activity of the anterior lobe might then explain the absence or relative mildness of the polyuria in certain animals which otherwise fulfil the requirements for diabetes insipidus. Following this rather theoretical line of reasoning, if this deficiency were overcome by furnishing the necessary anterior lobe principles, polyuria should occur. This explanation would apply to our cats 12 and 13 and perhaps 10 and 11. It is an odd circumstance that the polyuria following anterior lobe administration is frequently prolonged. This is perhaps explained by assuming the resumption of function by anterior lobe remnants, for it is almost inconceivable that effects produced by a course of injections of glandular material lasting a few days should persist for weeks afterward.

It is clear from our data, scant though it is, that polyuria can be produced in cats in early pregnancy and that it may persist throughout the gestation period, with possibly some diminution shortly before the onset of labor. This diminution amounted to about 40 to 50 per cent in cat D121, which does not, of course, compare with the total disappearance of polyuria before parturition in the human case of Duvoir, Pollet and Cachin. Unfortunately, in 2 of

our cats, D38 and D48, pregnancy was terminated more or less accidentally and neither cat survived. Of the 2 cats, D121 and D61, 1 with marked polyuria and 1 with mild, both came to term, but neither was able to complete delivery and data as to the course of the polyuria following parturition are lacking. It is hardly worth while at this time to speculate as to the reasons for the abnormal labor in these cats, but the depression of the water exchange in cat D121 and in the case of Duvoir, Pollet and Cachin comes more directly within the scope of our present interest. The latter authors also found it possible to reduce the polyuria in their patient with large doses of folliculin. Similar results have been obtained by Troisier (20) and Beltrametti (21). None of these writers offer a direct explanation for these effects, but imply that they are due to some indirect influence through the hypophysis, and Troisier points out that any such influence must be a transitory one in view of the prompt reappearance of the polyuria when the folliculin treatment is suspended. Of some interest in this connection are the findings of Moore and Price (22) and Nelson and Overholser (23), who report that prolonged estrin or testis-extract injection may have an inhibitory effect upon the anterior pituitary. If it is true that during pregnancy estrin is in some way held in check but gradually regains its influence toward the time of parturition (24), there may be some reason for supposing that increased estrin activity toward this time may be concerned with the diminution of polyuria as reported. It is clear that more experimental work along these lines is needed before an adequate explanation will be forthcoming.

SUMMARY

The observation previously made, that thyroidectomy may somewhat reduce but does not abolish the polyuria produced by lesions of the supraopticohypophyseal system in cats, has been confirmed in 4 other cats. Feeding dessicated thyroid to such animals elevated the water exchange equal to or exceeding the level existing before thyroidectomy. Castration performed on 4 cats with diabetes insipidus failed to diminish the polyuria, and in 1 of these the water exchange increased after ovariectomy.

Administration of a crude anterior lobe preparation, while ineffective in producing polyuria in normal cats, may increase the water exchange in cats with a tendency to diabetes insipidus. It was not invariably effective, however, and cats already displaying marked polyuria may not respond to such treatment.

Cats operated in the early stages of pregnancy may develop marked polyuria which persists, with some indication of diminution immediately before parturition. In the few cases available at present, parturition has been apparently abnormal, has not been completed and has ended fatally for the mother cat. This has prevented observation as to the course of the polyuria after parturition.

REFERENCES

1. Fisher, C., W. R. Ingram, and S. W. Ranson: Arch. Neurol. & Psychiat. **34:** 124. 1935.
2. Fisher, C., W. R. Ingram, W. K. Hare, and S. W. Ranson: Anat. Rec. **63:** 29. 1935.
3. Ingram, W. R., C. Fisher, and S. W Ranson: Arch. Int. Med. **57:** 1067. 1936.
4. Fisher, C., and W. R. Ingram: Endocrinology **20:** 762. 1936.
5. Ingram, W. R., and C. Fisher: Anat. Rec. **66:** 271. 1936.

6. Fisher, C., and W. R. Ingram: Arch. Int. Med. **58:** 117. 1936.
7. Schockaert, J. A.: Anat. Rec. **50:** 381. 1931.
8. Mahoney, W., and D. Sheehan: Am. J. Physiol. **112:** 250. 1935.
9. Schlotthauer, C. F.: J. Am. Vet. Med. Assoc. **39:** 673. 1935.
10. Teel, H. M.: J.A.M.A. **93:** 760. 1929.
11. Barnes, B. O., J. F. Regan, and J. G. Bueno: Am. J. Physiol. **105:** 559. 1933.
12. Biasotti, A.: Compt. rend. Soc. de biol. **115:** 329. 1934.
13. Gaebler, O.: Am. J. Physiol. **110:** 584. 1935.
14. Duvoir, M., L. Pollet and M. Cachin: Bull. et mém. Soc. méd. d. hôp. de Paris. **48:** 1444. 1932.
15. Marine, D.: Physiol. Rev. **2:** 520. 1922.
16. Swartz, J. L., and R. L. Thompson: J. Med. Res. **24:** 299. 1911.
17. Nicholas, J. S., and W. W. Swingle: Am. J. Anat. **34:** 469. 1925.
18. Davis, D. J.: Arch. Path. **4:** 937. 1927.
19. von Hann, F.: Frankfurt. Ztschr. f. Path. **21:** 337. 1918.
20. Troisier, J.: Bull. et mém. Soc. méd. d. hôp. de Paris. **48:** 1451. 1932.
21. Beltrametti, L.: Endokrinologie. **16:** 241. 1935.
22. Moore, C., and D. Price: Am. J. Anat. **50:** 13. 1932.
23. Nelson, W. O., and M. D. Overholser: Endocrinology. **20:** 473. 1936.
24. Robson, J. M.: Edinburgh M. J. **43:** 395. 1936.

THE EFFECT OF ADRENALECTOMY AND THYROIDECTOMY ON KETONURIA AND LIVER FAT CONTENT OF THE ALBINO RAT FOLLOWING INJECTIONS OF ANTERIOR PITUITARY EXTRACT[1]

EDITH G. FRY

From the George S. Cox Medical Research Institute, University of Pennsylvania
PHILADELPHIA, PENNSYLVANIA

It has been demonstrated that injections of neutralized alkaline extracts of the anterior pituitary produce marked acetonuria in albino rats maintained on diets high in butter fat (Burn and Ling, 1), or fed sodium acetoacetate by stomach tube (Butts, Cutler and Deuel, 2). The latter group as well as Black, Collip and Thomson (3) also observed that anterior pituitary extract greatly augmented the slight ketonuria of the normal fasted rat.

The adrenal gland was suggested as a mediating factor in the effects of the ketogenic principle of the anterior hypophysis by Steppuhn (4), but no experimental data were given. Recently, Long (5) found hypophysectomized-depancreatized cats, treated with Squibb's APE produced large amounts of urinary acetone bodies, although before injection they had excreted negligible amounts (6). When the same extract was given to adrenalectomized-depancreatized cats, acetonuria was either absent or very slight (5). The presence of the adrenal gland seemed essential for the ketogenic effect of the extract. Rietti (7), however, had observed no suppression of ketonuria after injections of APE to dogs whose adrenals had been denervated or whose adrenal medullae had been removed. However, there remained the adrenal cortex for consideration.

Evans (8) noted that phloridzin produced only one-sixth as much acetonuria in adrenalectomized rats as in normal ones, whereas rats bilaterally demedullated developed more than half the amount formed by intact rats. Black (9) noted rather variable responses among adrenalectomized rats with thyreotropic extract. The frequent occurrence of accessory adrenal tissue was suggested by Collip (10) to account for the variability.

EFFECT OF ADRENALECTOMY ON ACETONE EXCRETION FOLLOWING INJECTIONS OF APE

It has been the purpose of the present research to ascertain whether the ketonuria produced in the fasted albino rat by injections of crude extracts of the anterior pituitary is the result of an action of the ketogenic principle on the suprarenal cortex. Since it has been found that 1 in 4 rats in our colony fails to respond to active APE, because of a natural immunity to the ketogenic sub-

[1] A preliminary report was presented to the American Physiological Society at Washington, D.C., March, 1936.

stance, every rat was made its own control. Each was injected before and after adrenalectomy in order that negative post-operative results might be significant.

Experimental. Immature rats of both sexes ranging from 40 to 60 days of age were fasted 3 days in wire metabolism cages supplied with glass drinking bottles. The cages were set in glass funnels in which circular pieces of fine wire were placed to collect the feces. The urine was collected in 100 cc. beakers containing 20 per cent $CuSO_4$ for preservation.

Rats that were to be maintained on NaCl after adrenalectomy received physiological saline or Rubin and Krick's (11) salt mixture during the pre-operative as well as post-operative period; those that were maintained on cortical extract after adrenalectomy received water before and after the operation.

Analysis of the entire 24-hr. volume of urine for total acetone bodies was made by Van Slyke's (12) gravimetric method. Changes in the preparation of the copper-lime filtrate were made as follows. Urine was collected directly in 20 cc. of 20 per cent $CuSO_4$, diluted to 100 cc. with distilled water and shaken with 3 gm. of Merck's calcium hydroxide powder. The remainder of the determination was carried out in the usual manner. The ketonuria was expressed as mg. of acetone per 100 gm. of body weight per day. Figures recorded represent the average daily excretion of the 2-day injection period.

Two preparations of APE were employed, one made by the Squibb Laboratories, and the other prepared from ox pituitaries by the method of Burn and Ling (1). At the beginning of the second and third days of fasting, 0.5 cc. of either extract per 100 gm. of body weight was injected subcutaneously and 2.0 cc. of physiological saline were given intraperitoneally to assure good urinary volume. The acetone excretion of normal uninjected fasting rats averaged 1.5 mg. per 100 gm. per day.

Additional controls were necessary on account of the presence of preservatives in the extracts used, which if excreted in small quantities depress the oxidation of β-hydroxybutyric acid, and if eliminated in large quantities increase the amount of precipitate formed (12). Ten per cent alcohol was used as a preservative in the Burn and Ling extract and in the cortical extract prepared by the Upjohn Company. One-half per cent phenol was used in the Parke Davis cortical extract, 'Eschatin.' Control injections of physiological saline containing 10 per cent alcohol given to 7 normal fasting rats resulted in an average excretion of 2.6 mg. acetone per 100 gm. body wt. per day, while similar injections of saline, containing 10 per cent alcohol and 0.5 per cent phenol, corresponding to the extracts administered, averaged 0.5 mg. per 100 gm. body wt. per day for a group of 5 rats. To insure, before and after adrenalectomy, the same increment or depression of the true ketone value arising from alcohol or phenol excretion, the rats of the cortical extract series were given during the pre-operative experiment, injections of alcohol-saline or phenol-saline in addition to the Burn and Ling pituitary extract.

Bilateral adrenalectomy under nembutal anesthesia was performed in a one-stage operation as soon after the preliminary injection period as the pre-fasting

weight was regained. Post-operative therapy consisted of saline mixtures for drinking fluid as mentioned earlier, or subcutaneous injections of adrenal cortical extract. The operated rats required 0.25 cc. twice daily of Upjohn's preparation and 0.5 cc. twice daily of 'Eschatin' to produce a gain in weight. All rats studied recovered quickly from the operation, and ate and drank well. In no case were there signs of adrenal insufficiency at the time of the post-operative administration of APE.

Saline or cortical extract therapy was discontinued immediately following the second injection of APE. The early death of the animal was selected as a

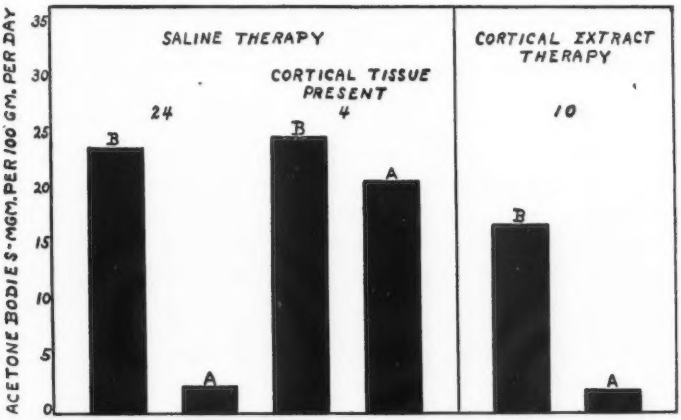

Fig. 1. Effect of APE on ketonuria of fasting rats before (*B*) and after (*A*) adrenalectomy.

criterion of complete adrenalectomy. Continued survival and growth was considered a certain indication of the presence of accessory adrenal tissue even though such tissue was not always found at autopsy.

Results. The ketogenic principle of APE which produced a marked ketonuria in the fasted rats of the saline group before adrenalectomy was completely ineffective after the removal of the suprarenal glands in the majority of the animals studied. The suppression of the normal response in the absence of the adrenals is shown graphically in figure 1 where the figures from injection of Burn's and Squibb's extracts in 24 rats of both sexes have been averaged together. Positive results after adrenalectomy were represented by only a small percentage of the preoperative response. Four rats which did not die within 14 days of saline withdrawal, gained weight and excreted ketone bodies in response to additional injections of APE comparable to the normal response of each rat (figure 1). Autopsy in each case revealed a well formed accessory body containing only cortical cells, enclosed in a capsular layer.

The same failure of APE to produce ketonuria was noted in adrenalectomized rats adequately maintained on either Upjohn or Parke Davis adrenal cortical extract. The results from experiments made with both adrenal cortical extracts have been averaged together under the column marked cortical extract

in figure 1. As soon as this therapy was withdrawn, gain of weight ceased and all died within 6 days except one which continued to gain weight and excreted some acetone bodies when tested with APE. No adrenal tissue was located at autopsy, but here again long survival seemed to indicate the presence of adrenal cortical tissue.

In a recent publication of Best and Campbell (13) injections of APE were shown to increase the liver fat content of the rat at the expense of the depot fat. Since acetone body excretion of the rat in response to the extract was inhibited in the absence of the suprarenals, and since hypophysectomy or adrenalectomy prevented liver fatty infiltration of the depancreatized cat (14), it seemed advisable to study what effect adrenalectomy would have on the mobilization of depot fat and fatty infiltration of the liver following administration of extracts of the anterior hypophysis.

EFFECT OF ADRENALECTOMY ON LIVER FAT CONTENT FOLLOWING APE

A study therefore was made of: *a)*, the liver fat content of the normal rat following injections of APE prepared by both the Burn and Ling (1) and Best and Campbell (13) methods; *b)*, the liver fat content of the completely adrenalectomized rat following a dose of APE effective in normal rats; *c)*, the liver fat content of rats with demedullated adrenals following anterior pituitary injections.

Experimental. The experimental animals ranged in weight from 90 to 160 gm. All rats were fasted 24 hours before experiments were begun. A control series of normal rats were injected subcutaneously with 3 cc. of physiological saline solution and the liver fatty acids determined. Extracts prepared according to Burn and Ling were administered in 0.5 cc./100 gm. of body weight with collections of material at 5 and at 24 hr. following a single injection, and at 6 and 24 hr. after a second successive dose. The observations a few hours after the dose were intended to detect any fatty infiltration which might occur immediately in response to APE, but which might have disappeared 24 hr. later (4). Larger doses (3 cc. =◇= 600 mg. original glands) of the extract were given to another group on each of 2 successive days. Finally 3 cc. of APE made by Best and Campbell's method were administered to normal rats, completely adrenalectomized rats and to demedullated rats.

The livers were removed at the end of the experiment, the excess blood was absorbed by blotting on filter paper, the entire organ was weighed quickly, and approximately a 400 mg. sample was analyzed by a modified Stoddard and Drury method (15) for total fatty acids. Total fats were estimated by evaporating an aliquot of the alcohol-ether extract to dryness in a tared beaker and weighing the residue. Parallel urinary ketone body determinations were made. Total fatty acids and total fats were also estimated on the kidneys of some of the animals.

Adrenalectomized animals were maintained on physiological salt solutions. Demedullation of the adrenals was performed in the manner described by Evans (16). It is advisable to inject adrenal cortical extract for several days

after this operation. These injections can then be discontinued.

Results. The saline control series showed an average liver fatty acid content of the normal fasted rat of 3.1 gm. per cent with a range of 2 to 4 gm. per cent. The ketone body excretion averaged 2.9 mg./100 gm. body weight.

TABLE 1. LIVER FAT AND ACETONE BODY EXCRETION OF VARIOUS FASTING RATS FOLLOWING SUBCUTANEOUS INJECTIONS OF BEST AND CAMPBELL'S ANTERIOR PITUITARY EXTRACT.

Rat No.	Injection	Liver				Urine
		Fatty Acids		Total Fats		
		%	tot. mg.	%	tot. mg.	Acetone Bodies mg./100 gm. body wt.
		A. Saline Controls				
82M	3 cc. saline	3.5	143	—	—	3.6
83M	" " "	4.2	191	—	—	2.5
84M	" " "	2.6	115	—	—	2.5
85M	" " "	1.9	67	—	—	2.9
86M	" " "	3.5	247	—	—	2.5
89M	" " "	2.7	99	—	—	3.3
Average		3.1	144	—	—	2.9
		B. Normal				
94M	3 cc. APE	6.8	405	9.6	594	18.6
95M	" " "	7.0	480	9.4	644	18.9
96M	" " "	6.5	391	8.6	522	8.5
97M	1.5 " "	7.2	431	8.5	565	11.9
Average		6.9	427	9.0	581	14.5
		C. Adrenals Removed				
98M	3 cc. APE	3.4	159	5.8	272	Q.N.S.
99M	" " "	3.4	149	6.6	292	2.7
100M	" " "	1.8	113	3.8	239	Q.N.S.
101M	" " "	2.9	130	4.4	200	1.0
86F	" " "	4.1	202	7.2	356	1.0
107F	" " "	3.7	151	7.0	282	7.0
Average		3.4	151	5.8	274	1.9
		D. Adrenals Demedullated				
102M	3 cc. APE	5.7	293	8.3	426	12.6
103F	" " "	5.7	341	8.3	495	13.9
104F	" " "	7.1	462	9.2	601	16.9
105F	" " "	7.3	387	10.0	529	14.1
108F	" " "	6.3	279	10.2	453	44.0
Average		6.4	352	9.2	501	21.1

The extracts prepared according to Burn and Ling, whether administered in 0.5 cc./100 gm. doses to fasted rats on 1 or on 2 successive days failed to raise the liver fatty acid content above the normal level, although the excretion of ketone bodies was considerable. Even observations made within a few hours of injections failed to disclose any increase in liver fat. However, a ketonuria was observed.

Large amounts (3 cc.) of these preparations on 2 successive days also failed to cause fatty infiltration of the liver. No ketonuria was produced and the rats were evidently suffering from the toxic effect of the extract.

On the contrary, one injection of the Best and Campbell extract produced a definite increase of the liver fatty acids in normal 24 hr. fasted rats. The average was 6.9 gm. per cent (see table 1). Total fats averaged 9.0 gm. per cent. The acetone body excretion averaged 14.5 mg./100 gm. The same dose of the Best and Campbell extract administered to adrenalectomized rats was ineffective in elevating the content of liver fatty acids and total fats. The range here varied from 2 to 4 gm. per cent as in normal saline controls. Ketone bodies excreted averaged but 1.9 mg./100gm. (table 1).

The series of demedullated rats when injected with 3 cc. of Best and

TABLE 2. EFFECT OF THYROIDECTOMY ON THE ACETONE BODY EXCRETION OF FASTING RATS FOLLOWING SUBCUTANEOUS INJECTIONS OF BURN AND LING ANTERIOR PITUITARY EXTRACT, 0.5 CC./100 GM. BODY WT.

Rat No.	Normal	Acetone Bodies (mg./100 gm./da.) After Thyroidectomy					
		3 da.	7 da.	14 da.	36 da.	48da.	52 da.
1M	0.2	25.5	—	—	11.2	—	—
3M	9.8	12.6	—	—	—	—	—
4M	11.4	0.6	—	—	—	—	—
8F	4.2	1.7	—	2.7	—	10.5	—
10F	22.1	9.3	—	28.8	—	19.1	—
11F	11.6	13.1	—	0.6	—	1.5	—
12F	14.2	4.9	—	1.2	—	9.4	—
15M	14.4	—	1.0	—	19.3	—	—
16M	11.2	—	1.3	—	5.9	—	—
18F	21.4	—	—	—	—	—	43.3
21F	22.9	—	—	—	—	—	16.6
22F	31.3	—	—	—	—	—	10.2
23F	16.3	—	—	—	—	—	13.1

Campbell's extract again revealed an elevated fatty acid content of the liver averaging 6.4 gm. per cent and 9.2 gm. per cent total fats (table 1). Ketone body excretion was even higher than the average in the normal rat group. At autopsy each rat, purposely demedullated, contained a regenerated piece of adrenal tissue. Histological examination confirmed the presence of adrenal cortical cells and no medullary tissue could be seen.

Although the presence of the adrenal cortex has been found necessary for the production of ketonuria in response to APE injection, the possibility of mediation of this action through the thyroid has not been excluded. Rietti (7) found no increase in ketonuria when thyroidectomized dogs were injected with the extract. Likewise, Magistris (17) in the case of rabbits, Eitel, Löhr and Loeser (18) working with rats, and Silberstein and Gottdenker (19), employing cats, found no increase in ketonemia following APE injections after removal of the thyroid gland. They concluded that the presence of the gland was essential for this action. On the other hand, Black, Collip and Thomson (3), working with thyroidectomized rats, obtained ketonuria with extract injections. Thyroxin had no noticeable effect on ketone excretion. They concluded that increased thyroid activity was not responsible for the ketogenic action. Best and

Campbell (13) from observations on rabbit blood acetone considered the time factor after thyroidectomy to be of importance and thereby explained the variable results of others. They obtained an increase in blood acetone for a month following operation, then a negative response.

EFFECT OF THYROIDECTOMY ON ACETONE BODY EXCRETION AND PHLORIDZIN FOLLOWING APE

We have attempted to show, *a)*, that the same rats which reacted well to anterior pituitary extracts, again eliminated acetone bodies at various intervals following thyroidectomy; *b)*, that the ketogenic action of phloridzin was not inhibited by removal of the thyroid gland.

Experimental. The rats were fasted and injected with Burn and Ling extract and the urine collected and analyzed as in the adrenalectomy experiments.

TABLE 3. EFFECT OF THYROIDECTOMY ON ACETONE BODY EXCRETION OF FASTING RATS FOLLOWING SUBCUTANEOUS INJECTIONS OF PHLORIDZIN.[1]

Rat No.	Acetone Bodies mg./100 gm./3 day.	Rat No.	Acetone Bodies mg./100 gm./3 day.
A. Normal		*B. After Thyroidectomy*	
1F	190.3	18F	183.2
2F	237.3	21F	187.6
3F	183.6	22F	115.0
4F	149.9	23F	152.9
Average	190.3	Average	159.7

[1] All rats received 50 mg. phloridzin in 1 cc. of olive oil daily.

Thyroidectomy was performed by the technique of Zeckwer (20). Occasionally the parathyroid glands were injured during the operation. Calcium lactate (6.5 mg. Ca/10 cc.) was supplied in the drinking fluid several days following thyroidectomy. If at any time during the experiment tetany developed due to permanently impaired parathyroid function, addition of calcium lactate to the drinking fluid relieved the symptoms.

Zeckwer (20) has shown that thyroidectomized rats continued to gain weight for 6 weeks following operation. The accumulation in weight was due to deposits of subcutaneous fat, rather than growth of bony structure. The kidney weights were an indication of stunted growth.

One series of thyroidectomized rats was thus injected several times with APE during the period of weight increase. Another was not injected until a weight plateau had been reached, averaging 6 weeks. At autopsy a generous piece of the trachea was removed and serial sections made to detect any remaining thyroid tissue. Sections were also made of the adrenal and pituitary.

Four female rats which responded to the ketogenic substance both before and 7 weeks after ablation of the gland were fasted and injected subcutaneously with 1 cc. of olive oil containing 50 mg. of phloridzin on each of 3 days. A normal series was given phloridzin simultaneously to test the potency of the chemical.

Results. The rats listed in table 2 varied considerably in the amount of ketonuria elicited by the extract, but the postoperative response was generally

equal to the pre-operative one. There was no correlation between the degree of ketonuria and the length of time elapsed since thyroidectomy. The phloridzinized-thyroidectomized rats (table 3) excreted quantities of ketone bodies comparable to the normal controls.

DISCUSSION

The absence of ketonuria in adrenalectomized rats was not the result of any immunity induced by the injection of APE before adrenalectomy, since normal rats given 2 injections separated by an equal interval of time responded with an equal degree of ketonuria on each occasion. Furthermore, the suppression of the fatty infiltration of the liver in the adrenalectomized group cannot be explained in this manner, since only 1 injection of APE was given. Even after 3 spaced injection periods, ketonuria still developed in most of the thyroidectomized rats tested.

In discussing the suppression of ketonuria in the adrenalectomized-depancreatized cat, Long and Lukens (6) suggested three possible explanations. *A)*. There existed an inadequate supply of the water and salt hormone of the adrenal cortex. The experimental fact that adrenalectomized rats receiving adequate amounts of this hormone still fail to exhibit increased acetonuria following pituitary extract would appear to dispose of this explanation.

B). Removal of the adrenals inhibits the normal function of the anterior pituitary. While this might be a factor in suppressing a ketonuria due to pancreatectomy, it does not appear to be an explanation for the failure of an animal to respond to injections of potent APE.

C). The anterior pituitary principle or principles that are concerned with the appearance of ketonuria are in some manner dependent upon the presence of adrenal cortical tissue. At the present time this appears to be the most logical explanation of several types of experiments concerning the influence of the adrenals upon ketonuria, such as the absence of the usual degree of ketonuria in the depancreatized cat whose adrenals have been removed (6) and the frequent failure of APE to elicit ketonuria from adrenalectomized-depancreaatized cats (5); the great reduction, if not suppression of ketonuria in phloridzinized fasted rats without adrenals (8); and the failure of fasted pregnant rats to excrete ketone bodies after adrenalectomy (21).

These experiments do not necessarily indicate that the injection of APE stimulates the adrenal cortex to produce a substance which in turn is responsible for the ketonuria observed, since it is entirely possible that the adrenal cortex normally secretes some substance, the presence of which is essential for the ketogenic activity of the anterior hypophysis. Whatever may be the manner in which the adrenal cortex is involved, it would appear certain that the water and salt hormone is not the governing principle.

The recent papers of Reichstein (22), Wintersteiner and Pfiffner (23, 24) and Kendall (25) have demonstrated the presence in the adrenal cortex of a variety of chemical compounds which at the present time are not thought to be concerned with the maintenance of the water and salt balance of the organism.

The importance of the adrenal cortex in the liver fat response to APE was

equally as striking as in the production of ketone bodies. Although Best and Campbell's figures for normal rats were higher, they gave 3 successive doses of the extract and their method of determination included cholesterol. However, the rise observed with one 3 cc. injection seemed adequate to justify our conclusion. It would appear that the adrenal cortex is intimately concerned with the phase of fat metabolism by which depot fat is transported to the liver.

Finally, if the trophic action of the ketogenic principle were mediated through the thyroid and loss of the adrenal cortex produced changes in the thyroid cells, failure of ketonuria after adrenalectomy would be expected. Positive results obtained repeatedly after thyroidectomy were convincing enough to exclude this gland as the indirect cause of ketonuria in response to anterior pituitary extract.

SUMMARY

Adrenalectomy completely suppressed the excretion of acetone bodies following the injection of anterior pituitary extract. The presence of adrenal cortical tissue permitted this ketogenic response to APE. Adrenal cortical extract therapy did not replace the ketogenic function exhibited by adrenal cortical tissue. Adrenalectomy prevented fatty infiltration of the liver in response to Best and Campbell's APE. Demedullation of the adrenals did not suppress the accumulation of liver fat in response to the same extract. Thyroidectomy did not suppress the ketogenic response to APE, or prevent the ketogenic action of phloridzin.

I wish to express my appreciation for the excellent direction and advice of Dr. C. N. H. Long in this work, and am greatly indebted to Margaret G. Gerstley for her assistance with the liver fat analyses.

I also wish to thank the Squibb and the Upjohn Laboratories for their generous supply of extracts for this study.

REFERENCES

1. Burn, J. H., and H. W. Ling: Quart. J. Pharm. & Pharmacol. **6:** 31. 1933.
2. Butts, J. S., C. H. Cutler, and H. J. Deuel, Jr.: J. Biol. Chem. **105:** 45. 1934.
3. Black, P. T., J. B. Collip, and D. L. Thomson: J. Physiol. **82:** 385. 1934.
4. Steppuhn, O.: Wien. Arch. f. inn. Med. **26:** 87. 1934.
5. Long, C. N. H.: Ann. Int. Med. **9:** 166. 1935.
6. Long, C. N. H., and F. D. Lukens: J. Exper. Med. **63:** 465. 1936.
7. Rietti, C. T.: Compt. rend. Soc. de biol. **117:** 57. 1934.
8. Evans, G. T.: Proc. Soc. Exper. Biol. & Med. **32:** 1246. 1935.
9. Black, P. T.: Thesis for Ph.D., McGill University. 1935.
10. Collip, J. B.: Ann. Int. Med. **9:** 150. 1935.
11. Rubin, M. I., and E. T. Krick: Proc. Soc. Exper. Biol. & Med. **31:** 228. 1933.
12. Van Slyke, D. C.: J. Biol. Chem. **32:** 455. 1917.
13. Best, C. H., and J. Campbell: J. Physiol. **86:** 190. 1936.
14. Long, C. N. H., F. D. W. Lukens, and E. G. Fry: Am. J. Physiol. **116:** 96. 1936.
15. Stoddard, J. L., and P. E. Drury: J. Biol. Chem. **84:** 741. 1929.
16. Evans, G. T.: Am. J. Physiol. **114:** 297. 1936.
17. Magistris, H.: Endokrinologie **11:** 176. 1932.
18. Eitel, H., G. Löhr and A. Loeser: Arch. f. exper. Path. u. Pharmakol. **173:** 205. 1933.
19. Silberstein, F., and F. Gottdenker: Klin. Wchnschr. **13:** 1434. 1934.
20. Zeckwer, I. T., L. W. Davison, T. B. Keller, and C. S. Livingood: Am. J. Med. Sc. **190:** 145. 1935.
21. Mackay, E. M., and R. H. Barnes. Proc. Soc. Exper. Biol. & Med. **34:** 682. 1936.
22. Reichstein, T.: Helv. Chim. Acta. **19:** 29. 1936.
23. Wintersteiner, O., and J. J. Pfiffner: J. Biol. Chem. **111:** 599. 1935.
24. Wintersteiner, O., and J. J. Pfiffner: J. Biol. Chem. **116:** 291. 1936.
25. Kendall, E. C., H. L. Mason, B. F. McKenzie, C. S. Myers, and W. D. Allers: Proc. Staff Meet., Mayo Clin. **10:** 245. 1935.

CLINICAL AND LABORATORY NOTES

A LIPOIDEMIA FOLLOWING ABORTION

ELDON M. BOYD*†

There are indications which might lead one to suspect that the completion of abortion in women would affect the concentration of blood lipids. There is a good deal of evidence that lipid metabolism is directly or indirectly under the control of most of the endocrine glands particularly those associated with sexual function.[1] A disturbance of endocrine balance by the sudden removal of the products of conception including the placenta, might well therefore alter lipid metabolism sufficiently to affect the percentage of lipids in blood.

During pregnancy in women there occurs a lipemia in blood plasma,[2] which is probably due more particularly to the deranged endocrine control rather than to a preparation for the proper nourishment of the fetus and is probably not due to a toxic factor.[3] From evidence obtained by many investigators, especially Gardner and Gainsborough, Hellmuth and Tyler and Underhill whose work in this connection has been previously reviewed,[2] it is known that the lipemia begins to appear about the end of the third month of gestation. The termination of pregnancy at this time would be logically assumed to result in a gradual return to normal in plasma lipid values. This phase of the subject has not been previously stressed; the author has noted that approximately 2 weeks after an abortion the blood lipids have returned to their normal values. The point being an obvious one, it does not seem necessary to present data to illustrate it.

In doing this work, the first sample of blood taken within 3 days of the completion of an abortion seemed to contain higher values for plasma lipids than one would ordinarily expect at the second or third month of normal pregnancy. Hence when sufficient data had been obtained, the results were compared with those of the corresponding period of normal pregnancy. The samples of blood were taken within a day or two of completion of abortion, either spontaneously or operatively induced, and a few in cases of incomplete abortion, most or all of which were probably induced. Plasma and red blood cells were analysed by the oxidative micromethods of Bloor as modified and used by the author in previous studies.

The results obtained in 16 cases of abortion in the second and third month and in 6 cases of normal pregnancy of the same duration have been summarized in the table. The standard deviation of the mean was calculated by a formula previously stated.[4] Patients aborting in the second and third month of pregnancy were found to contain, on the average, 30 per cent more total lipid in their plasma than cases of normal pregnancy of the same duration. This increase in total lipid was due to an increase in all of the component lipids but more especially of ester cholesterol,

* Department of Pharmacology, Queen's University, Kingston, Ontario, Canada.
† This work was aided financially by the Alice F. Richardson Fund of the Kingston General Hospital.
[1] Boyd, E. M., and C. A. Elden: Endocrinology, **19**: 559. 1935.
[2] Boyd, E. M.: J. Clin. Investigation, **13**: 347. 1934.
[3] Boyd, E. M.: Am. J. Obst. & Gynec. **32**: 937, 1936.
[4] Boyd, E. M.: Am. J. Obst. & Gynec. **30**: 323. 1935.

free cholesterol (and hence also of total cholesterol) and phospholipid, values for each of which were increased 40 per cent or more on the average. Compared with normal pregnancy of the same period, this lipemia of abortion is thus more strictly a lipoidemia, since one usually considers the lipoids as consisting of the lipids minus neutral fat, lipids being the all inclusive term.

It may be seen from the table that there were no significant changes in the lipid content of the red blood cells following the onset of abortion. The lipoidemia of abortion is thus relegated solely to plasma.

The peculiar feature of the changes recorded in the plasma lipids following abortion was the increase in the non-neutral fat lipids. In most lipemias, such as

THE LIPID COMPOSITION OF BLOOD FOLLOWING ABORTION. VALUES IN MG. PER 100 CC.

Condition and Value	Total lipid	Composition of total lipid					
		Neutral fat	Total fatty acids	Cholesterol			Phospho-lipid
				Total	Ester	Free	
PLASMA							
Abortion. *Min.*	630	55	349	110	40	33	156
Max.	766	408	562	207	151	87	293
Mean	717	243	456	170	111	59	229
Std. Dev.	±43	±97	±56	±34	±36	±16	±42
Normal Pregnancy 2–3 months. *Mean*	557	222	371	120	77	43	163
Std. Dev.	±55	±58	±47	±26	±31	±4	±26
RED BLOOD CELLS							
Abortion. *Mean*	622	71	338	171	25	146	358
Normal Pregnancy 2–3 months. *Mean*	644	81	336	158	26	132	353

those of pregnancy, diabetes, hemorrhage, nephritis and hypothyroidism, the typical finding is a marked increase in the neutral fat content of plasma and lesser increases in the other lipids. The apparently greater increases in the non-neutral fat lipids in abortion arise from the fact that the results in abortion were compared with those in early normal pregnancy and the non-neutral fat content of plasma was found lower in early normal pregnancy than in non-pregnant persons. The onset of the normal lipemia of pregnancy has been found in this work to be characterized by increasing values for neutral fat and decreasing values especially for ester cholesterol in plasma. This sequence again is typical of the onset of most lipemias which have been sufficiently studied. The peculiar lipopenic change in ester cholesterol has not been emphasized in the work of most other authors except in that of Gardner and Gainsborough noted above; it has been discussed by Boyd[5] in describing the lipemia of nephritis.

By comparing the means and standard deviation in the 2 groups of cases, it may be seen that there exists a significant difference in most of the values in abortion compared with those of the same period in normal gestation. For example, the mean minus the standard deviation for total lipid of plasma in abortion (717 — 43 = 674)

[5] Boyd, E. M.: Canad. M. A. J. 36: 18. 1937.

was considerably greater than the mean plus the standard deviation for total lipid of plasma in early normal pregnancy (557 + 55 = 612), indicating that more than 5 of 6 cases of abortion may be expected to contain more total lipid in plasma than more than 5 of 6 cases of early normal pregnancy.

The question arises, what is the cause of this lipoidemia of abortion? A significant observation in this respect seems to be the fact that 5 of the samples of blood were taken from cases of incomplete abortion, just before completion in hospital, and these 5 cases showed plasma lipid values just as high as those in the remainder of the group. The factors which one must consider as causes therefore are: *a)* bleeding, and *b)* the loss, partial or complete, of the influence of the early products of conception in the uterus. Bloor[6] has shown that persistent bleeding produces a lipemia in rabbits, but it has been usually found in the author's laboratory that anemia in man is accompanied by low values for plasma lipids and this also appears in most published reports. Since plasma lipid values were equally as high in cases of incomplete abortion wherein most of the placental tissue had been lost but bleeding had continued, it would appear that bleeding was probably a factor as well as, or rather than, the loss of the placental hormones. It would be futile at present to discuss further the possible cause of this lipoidemia of abortion.

Summary. Incomplete abortion in women, both before and shortly after completion, was found associated with an increase in plasma lipid values compared with those in the corresponding period of normal gestation, but no change in the lipid content of the red blood cells. The increase was seen chiefly in the ester cholestrol, free cholesterol and phospholipid fractions of the plasma lipids.

PITUITARY RESPONSE TO CERVICAL DILATATION

CHARLES H. BIRNBERG*

In October of 1934, the author published a series of cases[1] in which a slightly anomalous condition of the cervix associated with sterility was observed. These patients were subsequently relieved of their sterility by repeated gradual dilatations of the cervix. The results, at that time, were interpreted on the basis of the cure of the anomalous cervical condition. However, the well-known observations that in the rabbit ovulation takes place only during copulation, suggested to the author the possibility that the endocrines might in some way be involved. Working under this assumption, a series of prolan determinations in the urine was instituted before and after each dilatation.

Thirty cases have thus far been studied. The majority of the women were normal menstruating females with no complaints. There were 3 women who complained of sterility. Women were selected in whom a negative prolan reaction in the urine was determined before treatment was instituted. The purpose was to see if a prolan response might be elicited after dilatation. At the beginning of the series the urine was examined for prolan at any stage during the cycle depending upon when the patients were seen at the office, but an attempt was then made to elicit a prolan response after the patients had supposedly ovulated.

The theory behind this line of reasoning was in the supposition that stimulation of the cervix through dilatation might stimulate the pituitary gland by way of the sympathetic nerve pathway, which in turn, would secrete a sufficiently large amount

[6] Bloor, W. R.: J. Biol. Chem. 49: 201. 1921.

* From the Department of Female Sex Endocrinology in the Division of Obstetrics at the Jewish Hospital of Brooklyn.

[1] Birnberg, C. H.: J.A.M.A. 103: 1143. 1934.

Patient	Last menstrual Period Date	Expected Period Date	First prolan determination		Dilatation	Second prolan determination	
			Date	Result		Date	Result
1	8/3	8/31	8/7	0	8/9	8/10	+
2	8/10	9/7	8/15	0	8/17	8/18	+
3	8/14	9/11	8/19	0	8/21	8/22	+
4	8/27	9/24	9/1	0	9/3	9/4	+
5	9/1	9/29	9/6	0	9/8	9/10	0
6	9/6	10/4	9/11	0	9/13	9/14	+
7	9/17	10/15	9/21	0	9/23	9/24	+
8	9/28	10/26	10/2	0	10/4	10/5	0
9[1]	9/30	10/28	10/5	0	10/7	10/8	+
10	10/4	11/1	10/9	0	10/11	10/12	+
11	10/15	11/12	11/20	0	11/22	11/23	+
12	10/21	11/18	10/26	0	10/28	10/29	+
13	10/29	11/26	11/3	0	11/5	11/6	0
14	10/31	11/28	11/5	0	11/7	11/8	+
15	11/2	11/28	11/9	0	11/11	11/12	0
16	11/7	12/5	11/12	0	11/14	11/15	+
17	11/12	12/7	11/17	0	11/19	11/20	+
18	11/18	12/16	11/23	0	11/25	11/26	+
19	11/22	12/20	11/27	0	11/29	11/30	+
			POST OVULATORY				
20	11/26	12/24	12/13	0	12/15	12/16	+
21	11/30	12/28	12/18	0	12/20	12/21	0
22[1]	12/3	12/30	12/19	0	12/21	12/22	+
23	12/10	1/7	12/27	0	12/29	12/30	+
24	12/16	1/13	1/4	0	1/6	1/7	+
25	12/18	1/15	1/7	0	1/9	1/10	0
26	12/23	1/20	1/11	0	1/13	1/14	+
27[1]	12/30	1/27	1/18	0	1/20	1/21	+
28	1/2	1/31	1/22	0	1/24	1/25	0
29	1/3	1/31	1/23	0	1/25	1/26	+
30	1/8	2/5	1/30	0	2/1	2/2	+

[1] These patients were sterile, but are now pregnant.

of prolan to be found in the urine. If this reasoning was correct, we might then have an explanation for the successful treatment for sterility in so-called normal women, by cervical dilatation. Since this work was started, Friedgood and Pincus[2] have shown that stimulation of the superior cervical ganglion of a rabbit will bring about pituitary stimulation with resulting increased ovulatory changes.

Of the 30 cases studied, 23 gave a positive prolan response within 30 hours after dilatation. In 7 cases, no prolan response was obtained (see table). The presence of maturing follicles in immature female mice was taken as the critera for a positive prolan response. Microscopic slides of the ovaries were made to be absolutely certain of the changes which took place.

Conclusions. Following gradual dilatation of the cervix, a positive prolan response was demonstrated in the urine of 23 of 30 women within 30 hours. This response was present in the preovulatory and postovulatory phases of the cycle.

The probability that this prolan response might be a factor in relieving sterility in so-called normal women is suggested. Three women in the series who complained of sterility are now pregant.

I wish to thank Dr. Raphael Kurzrok for his help and encouragement.

191 Ocean Avenue
Brooklyn, New York

[2] Friedgood, H. B., and G. Pincus: Endocrinology 19: 710. 1935.

ASSOCIATION NOTICE

TWENTY-FIRST ANNUAL MEETING

The Twenty-first Annual Scientific and Business Meetings of the Association for the Study of Internal Secretions will be held in the Vernon Room of the Chalfonte-Haddon Hall, Atlantic City, New Jersey, Monday and Tuesday, June 7 and 8, 1937.

The Scientific Sessions will be held on Monday, June 7 at 9:30 A.M. and 2:00 P.M.; and on Tuesday, June 8 at 9:00 A.M. and 2:00 P.M. The annual subscription dinner will be held Monday evening at 7:00 o'clock.

The Annual Business Meeting will be held immediately following the Scientific Session on Tuesday afternoon.

Detailed information regarding the program, dinner, and the hotel accommodations at Atlantic City is being sent to all members of the Association. Others may secure this information by addressing the Secretary, Dr. E. Kost Shelton, 34 West Micheltorena St., Santa Barbara, California.

The final program of the Scientific Sessions will be published in the next issue of this Journal.

F. M. POTTENGER, *President*
E. KOST SHELTON, *Secretary*

CURRENT ENDOCRINE LITERATURE

COLLABORATORS

Fuller Albright
I. Andrussier
Wayne J. Atwell
Geoffrey Bourne
Israel Bram
John C. Burch
John C. Donaldson
Maurice H. Friedman
Murray B. Gordon
Arthur Grollman
Frank A. Hartman
E. C. Hamblen
F. C. Koch
Edward Larson
J. T. Lewis
Joseph M. Looney
Warren O. Nelson
J. P. Pratt
A. T. Rasmussen
Elmer L. Sevringhaus
L. B. Shpiner
D. L. Thomson
R. L. Zwemer

ADVANCE ABSTRACTS

A short test for ovarian follicular hormone and other estrogens. Allen, E., G. M. Smith and W. U. Gardner. To appear in this Journal.

A short test for estrogenic substances is described; it may be completed in 10 to 16 hours as compared with 48 to 56 hours for the vaginal smear test. It substitutes recognition of early growth produced by the estrogenic hormone in the vaginal wall for the complete estrous growth as required by the original test. This is made possible without sacrificing the clear-cut nature of the endpoint, by use of colchicine with the estrogenic material to be tested. Active estrogenic material starts mitotic division of the basal cells of the vaginal epithelium. Colchicine arrests the mitoses, thus accumulating the evidence of growth. Biopsy specimens of vagina are used instead of vaginal smears for determination of the endpoint.

Experimental acute hyperparathyroidism. 1. Chemical studies. Cantarow, A., J. T. Brundage and E. L. Housel. To appear in this Journal.

Studies of the blood during a state of acute severe hyperparathyroidism maintained over a period of 3 days in 6 dogs revealed the following: *1)*, an increase in serum protein concentration, due probably to hemoconcentration; *2)*, a practically proportional increase in both diffusible and non-diffusible fractions of serum Ca, reaching a maximum after 24-36 hours; *3)*, a terminal rise of inorganic P, the diffusible P remaining practically unchanged; *4)*, the diffusibility of serum Ca was not apparently influenced by changes in serum protein; *5)*, there was a striking initial diminution in corpuscular ester P, with a subsequent increase, due probably to the supervention of renal functional impairment; *6)*, the serum phosphatase increased steadily throughout the course of the experiment. The data suggest that: *1)*, the modes of action of the parathyroid hormone and vitamin D are not identical; *2)*, the increase in serum Ca resulting from parathyroid administration is not due entirely or even largely to the formation of a non-diffusible calcium compound.

Comparative action of gonad-stimulating hormones on the ovaries of rats. Fevold, H. L., F. L. Hisaw and R. O. Greep. To appear in this Journal.

The facts which have been presented offer additional evidence for the duality of the pituitary gonadotropic hormones. The two preparations affect the ovary in a characteristic manner, not only from a qualitative standpoint as has been reported but also the quantitative limits of the two singly and when combined are quite different. The action of FSH is quite sharply limited in its effect while that of FSH plus LH is not. The results further prove that tannic acid and copper salts do not alter the nature of the ovarian response to gonadotropic preparations in that the quantitative limits of the physiological reaction are not changed. This fact again differentiates the increased action due to these substances from the augmentation of FSH by the LH preparation.

Fatal functional uterine bleeding with report of a case. Hamblen, E. C., and D. H. Sprunt. To appear in this Journal.

A study of fatal uterine hemorrhage in a 15-year-old girl is described and the necropsy findings detailed. The endometrium was atrophic being characterized by a paucity of glands and widely dilated vessels. The ovaries showed cysts with degenerated granulosa and contained no corpora lutea. Critical estrin deficiency with impairment of vascular proliferation, tonicity and integrity is suggested as the responsible mechanism.

The action of pituitrin, estradiol and progesterone on the human uterus in vivo. Kurzrok, R., H. Wiesbader, M. G. Mulinos and B. J. Watson. To appear in this Journal.

Experiments on human uteri in vivo demonstrate that the uterus shows spontaneous contractions during all phases of the menstrual cycle. The uterus reacts to pituitrin in a positive manner during all phases of the cycle. There is no corpus luteum inhibition, hence the reaction offers no evidence as to the time of ovulation. Progesterone may occasionally produce an increase in tonus and amplitude of contraction. Progesterone produces uterine cramps during the postmenstrual phase of the cycle, but not during the premenstrual phase. The cramps are not associated with significant changes in the graph nor with a previous history of dysmenorrhea. Estradiol (benzoate) occasionally produces a rise in amplitude and tonus; it may produce cramps during the first half of the cycle, without significant changes in the tracing. Oily solutions of progesterone and estradiol are rapidly absorbed from the local site of injection, the effects being observed within 30 minutes.

Observations on carbohydrate metabolism following irradiation of the pituitary gland. Pijoan, M., and R. Zollinger. To appear in this Journal.

Carbohydrate metabolism studies were carried out on a group of 10 patients who were subjected to considerable irradiation of the pituitary body for the treatment of the menopausal syndrome. The ages of the patients varied from 29 to 50 years, with an average of 42 years. The studies were made before and after the irradiation. Each patient received a total of 1400 to 1600 R skin doses over a period of 4 days, without change in carbohydrate metabolism.

Cachexia hypophyseopriva (Simmonds' disease). Regester, R. P., and T. D. Cuttle. To appear in this Journal.

A case is presented of cachexia hypophyseopriva in an unmarried woman of 28 years. Clinical and laboratory findings show this to be a typical case although it was not proven by autopsy. Treatment was carried out with anterior pituitary extract (Squibb), the AP growth hormone, and the AP maturity factor, a total of 4570 R.U. being administered. Failure of this therapy to obtain beneficial results is recorded.

The effect of hypophyseal injection and implants on the activity of hypophysectomized rats. Richter, C. P., and J. F. Eckert. To appear in this Journal.

Hypophysectomy of 7 control females produced a marked decrease in spontaneous activity, food and water intake. Daily injections of emulsion of fresh anterior lobe substance started on the day of hypophysectomy greatly reduced the amount of the decrease in activity, food and water intake in 4 of 13 rats, but produced full replacement in only 1 animal. Hypophyseal transplants to the anterior chamber of the eye showed definite viability in 13 of 14 attempts, but had a small stimulating effect on activity, food and water intake only in 4 animals. Daily injections of fresh anterior lobe emulsion had the greatest effect on the ovaries, the least on the adrenals; implants to the eye had no effect at all on the ovaries but a definite effect on the adrenals. Replacement therapy to be complete must restore activity and food intake as well as body weight and the reproductive tract.

Pubertas praecox (macrogenitosomia). Rush, H. P., J. B. Bilderback, D. Slocum and A. Rogers. To appear in this Journal.

Three cases of typical hypergenitalism (macrogenitosomia) occurring in one family are reported. Male sex hormone was demonstrated in the urine of each of the 3 by use of the capon-comb test. Normally this is not found in boys under 10 years of age. Prolan could not be demonstrated by the action of urine-extract in immature female rats in any of the cases. No associated signs or symptoms were present from which to suspect tumor or marked hypertrophy of the pineal, adrenal cortex, gonads, pituitary or thymus. There were no signs or symptoms from which a cerebral lesion might be suspected. These cases seem to fall in the

group called primary, primary-constitutional, or congenital pubertas praecox. In addition to the 3 cases reported the family history disclosed 5 other instances in 4 generations. The condition seems, therefore, to have been genetically determined. It is possible that what was inherited may have been an abnormal responsivity to gonadotropic hormones.

Carbohydrate metabolism studies in hypophysectomized rats. The relation of various methods of glucose administration to the blood sugar curve. Samuels, L. T., and H. A. Ball. To appear in this Journal.

Rats hypophysectomized 2½ weeks or less when given 1 gm. of glucose per 100 gm. body weight by stomach tube yielded curves similar to those obtained in normal animals with the same dose. However, when this amount of glucose was given by stomach tube 5 weeks after operation a high curve of diabetic type resulted. In contradistinction to alimentary administration if the above dose or one-half the amount was given subcutaneously, abnormally high sugar curves resulted as soon as 1 week after hypophysectomy. This inability to remove excess sugar from the blood progressively increased in severity for a period of several weeks. This suggests an indirect effect of pituitary removal. A decrease of 36 per cent in the rate of intestinal absorption of glucose explains why the blood sugar curves in the animals which received the sugar in this way did not show higher than normal curves during the first 2½ weeks following hypophysectomy. This deficiency of intestinal absorption for glucose appears abruptly after pituitary removal and once it is established, remains constant. This suggests a direct effect of pituitary deprivation.

The effects of hypophyseal implants from normal mature guinea pigs on the sex organs of immature guinea pigs. Schmidt, Ida G. To appear in this Journal.

Hypophyses from normal sexually mature female guinea pigs were removed at different times during the estrous cycle and implanted into immature female guinea pigs. Single or double estrous implants were ineffective. Implants from donors 5 to 6 days after estrus caused little or no follicular stimulation, but a typical vaginal estrus and some uterine reaction. Implants from 7 to 10 days after estrus caused some growth of follicles, but no uterine or vaginal stimulation. Implants from donors 11 to 15 days after estrus caused rapid growth of existing follicles, vaginal estrus, enlarged uteri with mucosal estrous changes and marked proliferation of the smooth muscle. Following quadruple implants, estrous changes appeared in all cases, but were delayed after the estrous implants, and were most marked following proëstrous implants. In no instance were follicles (in the recipients) fully mature, no ovulation occurred and no corpora lutea were formed. Following a single male hypophyseal implant, mature follicles, which degenerated, were seen in the ovaries of the recipient. Following a double male implant, ovulation occurred and corpora lutea were formed.

The effects of hypophyseal implants from guinea pigs with irradiated ovaries on the sex organs of immature guinea pigs. Schmidt, Ida G. To appear in this Journal.

Following irradiation with a minimum sterilization dose of x-rays, the ovaries of some animals functioned normally but follicles were few, estrous cycles were slightly disturbed in length and vaginal smears were indefinite. Implants of these hypophyses produced responses similar to those following normal implants from corresponding stages of the estrous cycle. The normal structure of the majority of ovaries, following irradiation, was replaced by 'interstitial gland,' without follicles except for an occasional developing or growing one, and without corpora lutea. Estrous cycles occurred irregularly, with prolonged estrous periods characterized by excessive desquamation of cornified cells or of all types of cells associated with vaginal estrus. Theelin excretion in the urine was usually less than during a normal estrous period. A single normal estrous hypophyseal implant produced no response in recipients. Most (12) single implants, from donors with interstitial gland ovaries, in prolonged estrus, produced responses similar to those following single normal proestrous or late diestrous implants. A few implants (6) were much more potent, producing intense follicular growth and formation of corpora lutea, comparable to the response following single or double male hypophyseal implants (normal) and in one instance a response many times greater than this. Diestrous implants reacted as did normal implants from corresponding stages of diestrus.

A study of the metabolism of a dwarf under treatment with growth hormone. Struck, H. C., and S. A. Szurek. To appear in this Journal.

Growth hormone of the anterior pituitary was administered to a dwarf, and a study made of the nitrogen, calcium and phosphorus balance before, during and after the treatment.

Nitrogen was retained, but calcium and phosphorus balances remained essentially unchanged during and after the administration of the hormone. An increase in weight was the only change noted in the patient's condition.

ENDOCRINE GENERAL

Histone combinations of the protein hormones. Bischoff, F., Am. J. Physiol. **117:** 182. 1936.

The purpose of the research was to find protein precipitants which might be used clinically to delay resorption of parenterally administered protein hormones, i.e. insulin, prolan, and pituitary gonadotropic principle. A zinc-free, acid-soluable, thymus histone was prepared, which precipitates insulin on the alkaline side of the insulin isolectric point. Given intravenously this combination produces a blood sugar response approximating the original insulin (9 rabbits). Given intramuscularly, a marked decrease in activity is indicated when the assay is made in the conventional manner (6 rabbits). Prolonged hypoglycemia without shock is produced by larger doses (5 rabbits). Histone by combining, not with the active principle itself, but with contaminating proteins, which adsorb the pituitary gonadotropic principle produces a decrease in physiological activity when the assay is made in the conventional manner (6 rats). Complete recovery is indicated when zinc salts are added. The mechanism of the so-called pituitary inhibitors is thus explained. Material of low activity is separated from an active filtrate, when thymus histone is added to either prolan or pituitary gonadotropic preparations in the pH range of 6.0 to 8.0. Histone insulin has been tried in 11 cases of human diabetes. Its activity upon hypodermic injection is shown by its ability to control diabetes mellitus either when used alone or in conjunction with regular insulin, and the production of hypoglycemic shock after appropriate dosage. When injected daily, it shows a 'pooling effect' after 4 to 5 days necessitating reduction of total daily unitage. The blood sugar lowering action of a single dose is noticeable 24 but not 48 hr. after injection.—K. A. Brownell.

The effect of adrenaline on muscle glycogen in adrenalectomized, thyroidectomized and hypophysectomized rats. Collip, J. B., D. L. Thompson, and G. Toby, J. Physiol. **88:** 191. 1936.

Two to 3 month-old rats were anesthetized with sodium amytal (intraperitoneal); one gastrocnemius was removed and 0.25 mg. adrenaline per kg. was injected subcutaneously. Three hours later the other gastrocnemius and a portion of the liver were removed. Initial and final bloods were also taken for glucose analysis. There were 11 normal, 15 adrenalectomized (4-7 day post operative), 23 thyroidectomized (7-33 days) and 36 hypophysectomized animals (21-184 days). Of the last named group, 2 were injected with adrenotropic and 9 with thyrotropic hormone, while 4 received desiccated thyroid for 5 to 10 days before the experiment. The injection of 0.25 mg. adrenaline per kg. into normal non-fasted rats reduced muscle and liver glycogen and produced hyperglycemia. In adrenalectomized rats there was greater reduction of muscle glycogen but without marked hyperglycemia. Thyroidectomized rats had hyperglycemia, but little reduction of muscle glycogen. Long hypophysectomized rats resemble both types since muscle glycogen is not mobilized and hyperglycemia does not appear. Treatment with AP makes them more normal, with desiccated thyroid restored the muscle glycogen mobilizing, but not the hyperglycemic response.—R. L. Z.

Quantitative studies with the thyrotropic hormone. Cuyler, W. K., B. F. Stimmel and D. R. McCullagh, J. Pharmacol. & Exper. Therap. **58:** 286. 1936.

Using a thyrotropic hormone, 1 mg. of which would cause a marked decrease of thyroid iodin in 200 gm. guinea pigs, 4 mg. doses caused very small changes of the iodin content in the thyroid glands of 30 gm. rats. Ten mg. of this thyrotropic hormone per liter of the aquarium water, was sufficient to cause the metamorphosis of 51 of 60 toad tadpoles within 20 days. Using frog tadpoles, in the state just before the development of the front legs, it was found that the injection of 3 doses of 0.025 mg. caused a definite acceleration of metamorphosis.—E. L.

The intermediary metabolism of endocrine glands. Proteolysis and ammonia formation in the endocrine organs (Der intermediare Stoffwechsel in den Drüsen mit innerer Sekretion). Epstein, S., and E. Mima, Biochem. Ztschr. **281:** 339. 1935.

Because of the chemical relation between protein degradation products and adrenalin, thyroxin and insulin, the proteolysis and ammonia formation of adrenals (cortex and medulla separately) thyroid, pancreas and thymus were investigated at 37°C. and pH varying from 3.4 to

7.0. With the exception of the pancreas spontaneous proteolytic processes were found to be weak. With all glands investigated, proteolysis was more pronounced with increasing acidity. Short autolysis of pancreas, adrenal cortex and thymus glands of young animals at weakly alkaline pH produced ammonia.—F. Breuer.

Studies in histochemistry. VII The concentration of vitamin C in the thymus in relation to its histological changes at different stages of development and regression. Glick, D., and G. R. Biskind, J. Biol. Chem. **114**: 1. 1936.

The vitamin C concentration was determined by titration with 2.6 dichlorophenol indophenol, on sections of tissue cut from frozen bovine thymus glands of 18 animals at various ages. The concentration in the whole gland was found to fall within the limits of 0.35 to 0.52 mg. per gm. for all ages from the 3.6 month fetus to the calf. The thymus of the steer had a lower concentration, 0.23 to 0.36 mg. per gm. and that of the cow and bull had a still lower value 0.04 to 0.17 mg. per gm. These latter glands when corrected for the amount of fat and connective tissue gave values for the glandular cells approximately the same as for fetus or calf.—J. M. L.

Further observations on tumor of the pineal body. Horrax, G., Arch. Neurol. & Psychiat. **35**: 215. 1936.

Two additional cases of tumor of the pineal body are reported. In an adult, the growth was partially removed and verified at operation. Subsequent to operation and roentgen therapy the patient had remained well and in active life for 1½ years, with no return of symptoms of intracranial disturbance. In a boy of 10, the tumor was verified only by ventriculography. This child presented typical macrogenitosomia praecox. He had a period of normal activity for a year following decompression and roentgen therapy. After this therapy he became more normally youthful in appearance with considerable loss of facial and body hair but persistence of enlargement of the genitalia.—R. G. H.

The influence of pregnancy and delivery on the glands of internal secretion (Influence de la grossesse et de l'accouchement sur les glandes a sécrétion interne). Khavine, J. B., Rev. franç. d'endocrinol. **14**: 307. 1936.

Five case reports demonstrating the influence of pregnancy and delivery on pluri-glandular instability involving ovaries, thyroid and anterior hypophysis were presented. Some degree of hypothyroidism or hyperpituitarism were noted in all cases, indicated by menstrual disorders, frigidity, premature deliveries and stillbirths. Administration of 0.1 gm. of desiccated thyroid 2 to 3 times daily relieved symptoms in all cases.—H. L. Stewart.

Further investigations on the uterotonic effect of insulin (Weitere Untersuchungen über den uterotonischen Effekt des Insulins). Klaften, E., Zentralbl. f. Gynäk. **60**: 2834. 1936.

Various preparations of insulin were found to sensitize the guinea pig uterus leading to greater spontaneous contractions and to greater response to posterior pituitary extracts. Inactivation of insulin by heating with alkali prevented this. There are variations between commercial preparations which are attributed to some impurities in insulin. The effects are obtainable in virgin, gravid, and puerperal animals but not in castrates with atrophy. The white rat and white mouse react in like fashion, but the rabbit uterus fails to show this response. Without explanation of the clinical need, the author speaks of using insulin plus calcium gluconate injections for treatment of dysmenorrhea and irregular bleeding.—E. L. S.

The adequacy of the chemical theory of smooth muscle excitation. Rosenblueth, A., and W. B. Cannon, Am. J. Physiol. **116**: 414. 1936.

The authors confirm Monnier's and Bacq's observation that some drugs like yohimbine greatly diminish the sensitivity of smooth muscle to adrenalin but have comparatively little influence on the responses produced by sympathetic stimulation. This observation was considered by Monnier and Bacq as proof of the existence of a second type of mediation of impulses besides that through the production of a sympathetico-mimetic substance, but the authors explain it as the result of a decrease of the permeability of the smooth muscle cells. The attenuation of the response to adrenalin is supposed to be due to its slow penetration into the cells; the response to sympathetic stimulation on the other hand is less impaired because the mediator is assumed to be liberated inside the cells. The effects of cocaine and of denervation can be ex-

plained by an increase of permeability. These assumptions are supported by studies on the electrical responses of the nictitating membrane and of the pilomotors. The I wave, which is supposed to be due to a depolarization of the cell membrane, is increased by yohimbine, decreased by cocaine and by previous denervation.—Emil Bozler.

The effect of diets and various substances on the vitamin C content of some organs of the rat. Svirbely, J. L., Am. J. Physiol. **116:** 446. 1936.

Rats are able to synthesize vitamin C irrespective of the composition of the diet. Adequate amounts of vitamin B factors are essential to obtain normal values for the concentration of vitamin C in the organs of the rat. The utilization of ascorbic acid by the tissues of the rat is increased as the metabolic condition of the animal, under the influence of desiccated thyroid gland of a-dinitrophenol, is increased. The simultaneous administration of sodium fluoride and thyroid gland, at a level where either substance alone has no appreciable effect on the animal, decreases the vitamin C content of the organs. Rats at the point of collapse as the result of excessive thyroid feeding have a very low ascorbic acid content of the tissues. The vitamin C content of the liver is decreased by the administration of cincophen or etherization of the animals. The relative difference in concentration of ascorbic acid in the gut (small intestine) and liver of rats fed various diets indicates the important role played by the small intestine in yielding primary precursors for the synthesis of vitamin C. The adaptability of the gut to adjust itself to cope with experimental conditions, not normal to the ordinary life of the animal, as well as with variation in age is emphasized. Rats with fatty livers and possible disarrangement of hepatic function, as the result of exposure to carbon tetrachloride vapor, still synthesize appreciable amounts of ascorbic acid.—Author's Summary.

PANCREAS

Investigation of a new method of standardization of insulin (Versuch ainer neuen Standardisierungsmethode für Insulin). Stefl, J., Arch. f. exper. Path. u. Pharmakol. **181:** 617. 1936.

The author discussed the two methods in general use for the standardization of insulin. The technique for the new method was based on the suppression of the hypoglycemic convulsions produced in the test rabbit after the injection of the insulin to be tested by the administration of glucose. Such a technique enabled the investigator to differentiate the pure insulin effect from the convulsive action of other nonspecific substances in the sample tested. This method was shown to be as accurate quantitatively as the other methods now in use.—R. Cleveland.

Heredity of diabetes mellitus (Untersuchungen zur Frage der Erblichkeit des Diabetes Mellitus). Steiner, F., Deutsches Arch. f. klin. Med. **178:** 497. 1936.

From a familial investigation of the incidence of diabetes the percentage for parents was 4.1, 19.8 for siblings and 16.6 for offspring. If both parents were diabetics ¼ to ⅓ of the children have diabetes. A familial incidence of diabetes was noted in 35.1% of the cases.—E. L.

Transformation of acetoacetic acid by methylglyoxal (Die Unwandlung der Acetessigsäure durch Methylglyoxal). Stöhr, R., Ztschr. f. physiol. Chem. **240:** 23. 1936.

Both ketol and methyl-glyoxal, orally administered, increased liver glycogen in fasted rats, but completely failed to do so when 0.5 to 2 units insulin was given simultaneously.—D. L. T.

The relation of pancreatic juice to the fatty infiltration and degeneration of the liver in the depancreatized dog. Van Prohaska, J., L. R. Dragstedt, and H. P. Harms, Am. J. Physiol. **117:** 166. 1936.

The livers of 7 dogs deprived of pancreatic juice by means of total pancreatic fistulae were examined at autopsy 23 to 43 days after the operation. Three in which severe infection occurred showed moderate degeneration (not comparable to that found in depancreatized animals). The remaining 4 livers were normal. Ligation of the pancreatic ducts in 3 dogs failed to produce fatty degeneration of the liver, while oral administration of from 100 to 1000 cc. of fresh dog pancreatic juice daily failed to prevent degeneration of the liver in 9 totally depancreatized dogs. Experiments on 1 depancreatized dog showed that 25 gm. of fresh beef pancreas was sufficient to prevent liver degeneration while choline chloride in doses of less

than 1.0 gm. per day were ineffective in preventing fatty degeneration of the liver in experiments on 4 depancreatized dogs. (It was estimated that 25 gm. of pancreas contained only 60 mg. of choline.) Oral administration of 100 gm. of beef brain (2 dogs) and 100 gm. of beef liver (1 dog) was without effect in preventing liver degeneration. The authors conclude that the beneficial effect of pancreas feeding after pancreatectomy is probably due to some specific substance in the liver.—F. A. Hitchcock.

The fate of the sugar which disappears after insulin injections (Das Schicksal des verschwundenen Zuckers bei der Insulinwirkung). Vendég, V., Arch. f. d. ges. Physiol. **237:** 683. 1936.

Determinations were made of the sugar and fat content of the blood, the glycogen content of the liver and muscles, and the fat content of the liver of fasted dogs before and after the injection of glucose and insulin. The results indicate that insulin is concerned in the regulation of the fat metabolism. The apparent disappearance of glucose following insulin injections is due to the conversion of glycogen to fat in the liver.—A. G.

The etiology of abdominal pain in diabetic acidosis. Walker, H., Ann Int. Med. **9:** 1178. 1936.

The author presents a case of diabetic acidosis associated with severe abdominal pain. The essential laboratory findings were: blood sugar 444 mg. %; plasma CO_2 20.2 vol. %; urinary sugar 4 plus; acetone 4 plus; diacetic acid 2 plus. Immediately after admission the patient was given 50 U. of insulin and 1000 cc. normal saline intravenously. The abdominal pain immediately began to lessen and entirely disappeared within 45 minutes. Unfortunately neither plasma chlorides nor sodium were determined. The author discusses the abdominal pain on the basis of an alteration of chloride metabolism.—G. W. Thorn.

Spontaneous hypoglycemia. Wilder, R. M., Internat. Clin. **3:** 143. 1936.

Wilder suggests that spontaneous hypoglycemia might with clinical justification be termed paroxysmal hypoglycemia. The causes of this condition are reviewed and classified under 2 headings: Cases due to hyperinsulinism and those due to causes other than hyperinsulinism. Among the latter are diseases of the anterior pituitary and cachexia, insufficiency of the adrenals and thyroid, diseases of the liver, idiopathic hypoglycemia, hypoglycemia of infants of diabetic mothers, and hypoglycemia of infants of normal mothers. Treatment demands the administration of glucose, by vein preferably. Prevention of attacks is accomplished by giving small meals at frequent intervals. Physical exercise should be curtailed. If a circumscribed tumor of the pancreas is discovered it should be removed; if not, mechanical diminution of the incretory activity by partial resection of the pancreas may be considered. Wilder cites a case in which relief was secured by ligation, with a double strand of thick silk, of the body of the pancreas anterior to the division of the celiac axis and splenic artery.—I. B.

PARATHYROID

The relation of the parathyroid glands to urinary lithiasis. Barney, J. D., and E. R. Mintz, J. Urol. **36:** 159. 1936.

Blood calcium and phosphorus studies were made on 288 cases showing urinary lithiasis. Twelve of these were cases of hyperparathyroidism as proved by operation. The authors summarize their studies as follows: While hyperparathyroidism is responsible for between 4% and 5% of all cases of urinary stone, the percentage of stones in the presence of this disease may be almost 70%. It is also true that patients may show both bone and urinary tract pathology in about 38% of cases of hyperparathyroidism. This disease does not seem to be a factor in the production of bilateral lithiasis. The symptoms and signs of urinary lithiasis in parathyroid tumor do not differ from those due to other causes. After removal of the tumor stones do not recur. It is believed that no physical examination, especially if urinary lithiasis is present or suspected, is complete without a careful study of the calcium and phosphorus content of the blood.—J. C. D.

The influence of irradiated ergosterol and parathyroid extract upon the rate of disappearance of intravenously injected calcium chloride. Freeman, S., Am. J. Physiol. **115:** 701. 1936.

Healthy adult dogs maintained on a standard low calcium diet were injected intravenously with calcium and the rate of disappearance of the calcium from the blood stream found to be

constant. Administration of parathyroid extract or irradiated ergosterol produced a fasting hypercalcemia and decreased the rate of removal of calcium from the blood stream.—R. R. Durant.

Influence of viosterol and parathyroid extract on mineral metabolism in osteogenesis imperfecta. Hansen, A. E., Am. J. Dis. Child. **50:** 132. 1935.

The mineral balances were determined in 2 cases of osteogenesis imperfecta on a standard regimen known to favor growth and development in normal persons. The effects on the mineral metabolism of feeding large doses of viosterol and of subcutaneous injection of parathyroid extract were also studied. Under standard conditions both patients exhibited a deficiency in the retention of calcium, phosphorus and magnesium. No other abnormality was discovered in reference to other features of the mineral metabolism during this control period. The most significant response to large doses of viosterol was the induction of a negative balance for most of the minerals, notably for calcium and phosphorus. The metabolism of nitrogen and sulphur underwent little, if any change. The urinary output of calcium and phosphorus tended to increase while the fecal output was decreased. This influence persisted for several days after the medication was discontinued. Parathyroid extract likewise caused an excessive output of calcium, phosphorus, magnesium, potassium and sodium. Its immediate effect was more marked than that of viosterol but there was no sustained action. While the author cannot conclude from his studies that hyperfunction of the parathyroids is a factor in the pathogenesis of osteogenesis imperfecta, it is demonstrated that an extract of these glands or of large doses of the similarly acting viosterol, accentuates the disturbance already present and should not be employed therapeutically.—M. B. G.

A severe form of tetania parathyreopriva treated with Collip's parathyreoid extract. Herlihy, J. D., M. J. Australia **1:** 17. 1936.

A description was given of a female patient aged 25 years with rigor coma and incontinence of the urine and feces. Consciousness was regained following intramuscular injection of parathormone. High calcium diet combined with vitamin D was given, but the nausea and coma returned and relief was again obtained by the use of parathormone. The patient had previously been treated for hypothyroidism.—G. B.

Parathyroids: A new anatomic concept. Hermann, C. S., J.A.M.A. **106:** 1357. 1936.

The author explains the variation in the number of parathyroids found in any given specimen, describes the parathyroid nerves and gives a location that may be considered normal for the inferior parathyroid. On each side of the neck a branch of the inferior thyroid artery joins with a branch of the recurrent laryngeal nerve to form a 'stalk' to the parathyroid. Similarly a branch from the superior thyroid artery is joined by a branch from the superior laryngeal nerve to form a 'stalk' to the superior parathyroid. These four stalks are constant in number regardless of the number of parathyroids. Each of these 4 stalks described may lead to a single gland. On the other hand, from any of these 4 stalks 2 or more parathyroids may arise. It is in this manner that the number of parathyroids varies in different individuals. There is a definite nerve given off by the recurrent laryngeal nerve at the point at which it crosses the main branch of the inferior thyroid artery. This inferior parathyroid nerve accompanies the parathyroid artery and enters the hilus of the inferior parathyroid. It appears to supply no other structure. The superior laryngeal branch of the vagus divides into an internal and an external branch. The external branch, which is much the smaller, passes downward to supply the cricothyroideus muscle. From this external branch a fine filament passes downward as the superior parathyroid nerve and joins the company of the superior parathyroid artery. The recurrent laryngeal nerve and inferior thyroid artery vary considerably in their location and course, but of necessity they always cross. It is at this point of crossing that the stalk referred to is formed. This slender stalk is usually about 5 mm. long and the parathyroid body lies in the inverted V shaped niche formed by the artery and the nerve. Of the 44 definitely identified inferior parathyroids, 34 were found in exactly this location. The remaining 10 had stalks of variable length. Some were as long as 4 cm. These may be called ectopic glands and they were found in variable positions, depending, it seemed to the authors, on the pull exerted by the parathyroid vein, which is a tributary to the inferior thyroid vein. The glands, either in the normal habitat or in the ectopic position, were always very close to one of the inferior thyroid veins. Rarely was the inferior parathyroid in contact with the thyroid. It was, then, usually free from the thyroid, posterior to and below it, snugly enveloped in a rug of fat. The similarity between embedded adrenal and parathyroid in its fatty capsule constantly impressed itself on the authors.

The anatomic-histologic picture in experimental hyper- and hypoparathyroidism in the albino rat (Contributo al quadro anatomoistologico dell'iper-ed ipo-funzione sperimentale dell paratiroidi nel ratto albino). Molinari-Tosatti, P., and M. Pisa, Sperimentale, Arch. di biol. **89:** 509. 1935.

Ablation of thyroid and parathyroids causes only a slight decrease in size of the organs, the most evident being that of the spleen, without histological changes, however. Treatment of normal rats with parathormone causes increase in weight of the spleen and kidneys, alterations in the cartilage, calcification of arteries, hyperplasia and hyperemia of the spleenic pulp, and degenerative and inflammatory lesions, with calcification, in the kidneys.—A. E. M.

Asthma, its treatment by the Collip parathormone (L'asthme, son traitement par la parathormone Collip). Piassetsky, N., Rev. franç. d'endocrinol. **14:** 321. 1936.

On the basis that many symptoms are common to asthma, tetany and parathyroid deficiency, a predisposing factor to the development of the asthmatic complex was presumed. Sixty subjects, 34 men and 26 women, ranging from 17 to 59 years, were given hypodermic injections of 0.5 to 2.0 gm. parathormone daily or on alternate days, according to the gravity of the case, a total of 15 to 30 injections. Observations extended over a period of 10 months showed: completely relieved 40%; relieved 35%; improved 20%; no results 5%. The attacks were not so promptly relieved as with adrenalin; improvement was more gradual and stable. —H. L. Stewart.

Infantile tetany. A metabolic study. Pincus, J. B., and I. F. Gittleman, Am. J. Dis. Child. **51:** 816. 1936.

The authors report the clinical course and chemical findings in a case of tetany in a 7-weeks old boy. Administration of parathyroid extract was followed by cessation of the convulsions and increase in blood serum calcium content, decrease in serum phosphorus and an increase in urinary calcium and phosphorus. The feces after treatment showed a reduction in phosphorus but no change in calcium. A complete recovery has continued for 2 years.—M. B. G.

The effect of parathyroid hormone upon the serum calcium and calcium excretion of normal and adrenalectomized rats. Pugsley, L. I., and J. B. Collip, Biochem. J. **30:** 1274. 1936.

Adrenalectomized rats, receiving 1% NaCl as drinking water, are abnormally sensitive to parathormone, of which 20 units daily elevates the serum calcium to 17 mg.% and causes congestive haemorrhage in the gastro-intestinal tract; the urinary excretion of calcium is also greater than usual, but as usual rapidly returns towards normal in spite of continued injections. The peculiar behavior of these animals is partly, but not wholly, ascribed to the NaCl administration, which in normal rats increases urinary calcium excretion and increases sensitivity to parathormone. The calcium excretion of rats receiving NaCl is not further affected by adrenalectomy. The development of refractoriness to parathormone may be observed in young normal rats receiving a calcium-rich diet which maintains them in positive calcium balance throughout.—D. L. T.

The effect of complete renal insufficiency on the action of parathyroid hormone in the dog. Tweedy, W. R., R. D. Templeton and F. A. McJunkin, Am. J. Physiol. **115:** 514. 1936.

The serum calcium and inorganic phosphate were studied for 72 hours in dogs suffering complete renal insufficiency and receiving parathyroid extract. Changes in the serum calcium were so slight even with huge doses of the extract as to lead to the conclusion that mobilization of calcium is dependent on kidney function. Hypercalcemia was produced in 2 dogs before nephrectomy with no effect on the increase in serum inorganic phosphate. The absence of necrosis and calcification or venous engorgement and hemorrhage in the stomach led to the conclusion that complete deletion of kidney function protected the animals from overdosage with parathyroid hormone.—R. R. Durant

THYROID

The histology of the thyroid gland in pregnancy. Abbott, A. C., and J. Prendergast, Canad. M.A.J. **34:** 609. 1936.

Histological comparison of the thyroids of 103 pregnant cows with those of 95 non-pregnant animals of similar age indicates only a mild increase of activity during pregnancy.

Thyroid enlargement in young women, in Manitoba, is as common before as during pregnancy, and is probably due solely to iodine deficiency. The authors do not believe that there is evidence of any striking change in thyroid activity during pregnancy, and maintain that the limits of normal variation are wider than is commonly supposed.—D. L. T.

Non-thyroid substance with a thyroid action (Nichtschilddrüsenstoffe mit Schilddrüsenwirkung). Abelin, I., Arch. f. exper. Path. u. Pharmakol. **181:** 250. 1936.

The preparation of a protein substance by the addition of iodine is described in detail. The active portion of the preparation produces a physiological action similar to that of thyroxin.—R. Cleveland.

Therapeutic effect of total ablation of normal thyroid on congestive heart failure and angina pectoris. xviii. The cardiac output following total thyroidectomy in patients with and without congestive heart failure, with a comparison of results obtained with the acetylene and ethyl iodide methods. Altschule, M. D. and M. C. Volk, Arch. Int. Med. **58:** 32. 1936.

In 19 of 23 patients the velocity of blood flow was slowed when the cardiac output was low. In patients with congestive failure the velocity of blood flow was much slower than in those without congestive failure. In 3 instances the velocity of blood flow did not reflect accurately the work of the heart. The venous pressure, arterial pressure and vital capacity were not significantly altered after total thyroidectomy in the patients in this series. The measurements obtained for the same patients by the acetylene and ethyl iodide methods were compared. Reduction in the work of the heart was associated with clinical improvement. The data obtained in this study are in harmony with the concept that the relief obtained after operation in patients with angina pectoris is due principally to reduction of the work of the heart to or below an amount which it can do without the development of anoxemia. In patients with congestive failure the basal oxygen consumption after total thyroidectomy falls well below the point at which it merely balances the low cardiac output, and the cardiac output at rest coincidentally becomes reduced below the preoperative level. This makes it possible for the cardiac output in such patients to increase appreciably in response to work, so that the degree of activity may be increased without discomfort.—I. B.

Influence of glycine on depleted creatine reserves of skeletal and cardiac muscle in experimental hyperthyroidism. Bodansky, M., and V. Duff, Proc. Soc. Exper. Biol. & Med. **34:** 307. 1936.

In rats rendered hyperthyroid by daily thyroxin injections, neither the administration of glycine, nor of creatine, prevented the usual creatine depletion of cardiac or skeletal muscle. The daily administration of 1 gram of glucose resulted in a decreased creatine excretion, but did not produce any increase in the creatine content of the muscles.—M. H. F.

Syndrome of diffuse muscular hypertrophy in infants causing athletic appearance: Its connection with congenital myxedema. Debre, R., and G. Semelaigne, Am. J. Dis. Child. **50:** 1351. 1935.

The authors report 2 cases in babies of intellectual retardation, arrested growth, retarded development and general muscular hypertrophy tending to an athletic aspect accompanied in 1 case with muscular hypertonia. The condition of the second baby was transformed by thyroid treatment. Congenital athyroidism may cause this syndrome and the administration of thyroid extract is advised. Similar cases have been mentioned and reported by Wieland and DeLange.—M. B. G.

Weight of the thyroid gland and athero-sclerosis. Derngal, E. P., Lancet **230:** 1354. 1936.

Attention is called to the relative smallness of the thyroid glands in Icelanders. Thyroid weights observed averaged 14.9 gm. in 14 males (20-50 years old) and 11.3 gm. in 20 females. The average weight for 57 males and females over 20 years of age was 13.3 gm. Athero-sclerosis in the population is unusually rare. It is probable that the abundance of iodin in the food, soil, and air is the cause of both phenomena.—L. B. S.

The rôle of the thyroid in the calorigenic action of vitamin D^1. Deutsch, H., C. I. Reed, and H. C. Struck, Am. J. Physiol. **117:** 1. 1936.

Experiments carried out on 2 dogs show that after complete thyroparathyroidectomy large doses of vitamin D^1 produce no significant increase in the metabolic rate. Previous work has shown that the calorigenic action of vitamin D^1 on normal animals is not due to action on the parathyroids. The authors therefore conclude that the thyroid plays an important part in this reaction. Variation in metabolic rate roughly paralleled changes in body weight.—F. A. Hitchcock.

Cyclic changes in the thyroid during the separate sexual stages of the pigeon (Über zyklische Veränderungen der Schilddrüse in den einzelnen Geschlechtsphasen der Taube). Elterich, C., Endokrinologie **18:** 31. 1936.

A definite cycle of thyroid activity is described and related to sexual activity for pigeons of both sexes. Especially in female birds the thyroid presents the histological picture of greatest activity in the moulting and nursing periods, while it appears to be inactive in the pre-laying period. The other stages, such as the so-called interval, the laying time and the nesting period, are intermediate in activity. There seems to be a reciprocal relation between activity and weight of the thyroid. By using the azocarmine staining method two varieties of colloid were observed. In the stages of greater activity more basophil colloid was seen while in the resting stage and in stages transitional from greater to lesser activity acidophil colloid predominated.—W. J. A.

Concerning the relation of the sodium variations in blood serum in thyrotoxic conditions (Über das Verhalten des Natriumspiegels im Blutserum bei thyreotoxischen Zustanden). Feldmaus, B., Acta Med. Scandinav. **88:** 39. 1936.

The sodium content of the blood serum was determined by the method of Kramer and Gittleman in 22 patients with thyrotoxicosis, in 3 patients with non-toxic goiter and 4 normal individuals. The values fell within normal limits in each group and the sodium content of the serum is therefore worthless as a test of hepatic function in thyrotoxicosis.—J. M. L.

Mineral metabolism in experimental hyperthyroidism (Il ricambio minerale nell'ipertiroidismo sperimentale). Fenicia, M., Arch. per le sc. med. **60:** 747. 1935.

Prolonged administration of thyroxin causes in rabbits a constant increase of phosphates in the serum; calcium is not consistently higher and the changes in other electrolytes are variable. The pH remains unchanged but the alkali reserve is reduced. The water elimination through the kidneys varies, the feces have always a higher water content. The elimination of phosphate and sodium through the kidneys and intestine is always increased, while that of magnesium, calcium, potassium and chlorine in most cases is also increased. The balance of calcium, magnesium and phosphorous often turns negative.—A. E. M.

A case of hyperthyroid-parathyroidism (Une case de hyperthyroparathyreose). Fiessinger, N., and H. R. Olivier, Ann. de méd. **38:** 389. 1935.

The symptoms significant for hyperthyroidism were tachycardia, amenorrhea, palpitations and trembling. The diagnosis of parathyroid involvement was suggested by pains in the bones with multiple fractures, hypercalcemia and increased phosphatase in the serum. Irradiation brought considerable relief of the symptoms.—A. E. M.

The effect of thyroxine on milk secretion and on the phosphatase of the blood and milk of the lactating cow. Folley, S. J., and P. White, Proc. Roy. Soc., London, s. B., **120:** 346. 1936.

Daily injection of thyroxine into cows in declining lactation increases milk flow and production of milk fat and non-fatty solids, but decreases milk phosphatase concentration.—D. L. T.

Hypothyroidism complicated by diabetes mellitus. Greenwald, H. M., and W. S. Collens, Am. J. Dis. Child. **50:** 979. 1935.

A case report of a 7½-year old girl with a history of hypothyroidism since 4 months of age and polyuria and polydipsia for 8 weeks. An acute infection produced a flair-up in the diabetic condition.—M. B. G.

Control of hyperthyroidism following partial thyroidectomy by removal of unusually small amounts of thyroid tissue. Haines, S. F., and J. deJ. Pemberton, Arch. Int. Med. **57:** 1104. 1936.

As practiced at the Mayo Clinic, the operation of subtotal thyroidectomy, in which is preserved a postero-mesial portion of each lobe equivalent to from ⅙ to ⅓ of a lobe of normal size, is comparatively free from technical complications, such as parathyroid tetany and injury to the inferior laryngeal nerve. Within 2 to 4 weeks after operation the basal metabolic rate is usually within normal limits, and after the lapse of 1, 3 and 5 years, more than 90% of the patients consider themselves improved and present evidence to indicate that they are free from hyperthyroidism. It is estimated that the death rate for patients who have successfully undergone thyroidectomy is no greater than that for an average group of persons. It is also estimated that after the lapse of 5 years from 2% to 5% of the patients have sufficient evidence of recurrence of symptoms to warrant a secondary operation. Three cases are presented in which recurrence of exophthalmic goiter occurred when the remaining amount of thyroid tissue was so small as not to be palpable. In none of these cases was control of hyperthyroidism achieved by the administration of compound solution of iodine. A second operation in each case revealed such a small amount of thyroid tissue that not more than 1 gm. could be removed. Control of hyperthyroidism was accomplished in each case after the removal of this small amount of tissue.—I. B.

The ultraviolet absorption spectra of thyroxine, thyronine, tyrosine, di-iodotyrosine and thyroglobulin. Heidt, L. J., J. Biol. Chem. **115:** 223. 1936.

The absorption spectra were measured at 22° with a Judd-Lewis sector photometer backed by a quartz spectograph. No marked differences were found in the spectra sufficient to warrant identification of any of the compounds in the presence of the others. Denaturization of thyroglobulin except with acetone did not alter the curve significantly.—J. M. L.

Does calcium neutralize thyroxine? Hellwig, C. A., J. Lab. & Clin. Med. **21:** 1131. 1936.

The author was unable to confirm the work of Zondek and Reiter that addition of calcium chloride to the water prevents the increased rapidity of metamorphosis of tadpoles produced by adding thyroxin to the water. One hundred tadpoles served as controls; 100 had thyroxin alone added to the water; 100 had thyroxin and calcium chloride added.—F. A.

Sugars and hormonal regulators of sugar metabolism in relation to the activity of thyroxin. Hoffman, O., and F. Gudernatsch, Endokrinologie **18:** 96. 1936.

Various pentoses, hexoses, disaccharides and polysaccharides were administered, by immersion or injection, to anuran tadpoles of different species and ages. After a period of sugar administration, the animals were subjected to thyroxin or diiodotyrosine treatment. The object of the experiments was to determine to what extent the response to thyroxin or diiodotyrosine could be accelerated, retarded or otherwise modified through the influence of these sugars. The experiments have shown that the accelerating influence of thyroxin on the rate of development may become modified in the presence of different carbohydrates. Galactose and sucrose definitely retarded the thyroxin reaction; lactose, dl-mannose and d-mannose exerted slighter retardation. Glucose and glycogen definitely accelerated the thyroxin reaction. In the presence of ordinary mixed food, adrenalin definitely accelerated the thyroxin effect while insulin retarded it.—W. J. A.

Carbohydrate metabolism. Role of the thyroid gland. Johnston, J. A., Am. J. Dis. Child. **48:** 1015. 1934.

The following observations were made on 4 children, 2 with hyperthyroidism and 1 each with hypothyroidism and cretinism. Basal-metabolism determinations were followed by intravenous injection of 40 cc. of 50% solution dextrose and further determinations made at 60, 120 and 180 minutes later. With the urinary nitrogen and the respiratory quotient the

metabolic mixture was calculated from the data of Lusk. Dextrose oxidation curves were plotted. It appeared that there is no impairment of carbohydrate metabolism in hyperthyroidism; if glycogen stores are maintained, injected carbohydrate is oxidized more readily than in normal persons. The diminution of carbohydrates sometimes seen in hyperthyroidism is the phenomenon of 'starvation diabetes' resulting secondarily from increased glycogenolysis and accelerated metabolism. The increased oxidation of injected carbohydrates is interpreted as due to impaired glycogenolysis and the delay in oxidation in the cretin, despite adequate glycogen stores, as due to decreased glycogenolysis. There is a marked sparing action on proteins following medication with thyroid extract in the cretin, the result of an increased availability of glycogen stores and heightened oxidation of fat. A relationship exists between the release of water from the body and the mobilization of sugar.—M. B. G.

Study of follicle hormone excretion in hyperthyroidism (Studium des Follikelhormons bei Hyperthreosis). Jonas, V., and E. Markalous, Zentralbl. f. Gynäk. **60:** 2614. 1936.

The 24 hour urine excretion of estrogenic substance was studied by von Siebke extraction and tests made in castrate mice. Patients with thyrotoxicosis numbered 16, and are compared with only 2 normals. There seems to be a lower excretion of estrin in thyrotoxics, but not in proportion to the metabolic rate or to the disturbance in menstrual cycles. In 2 cases studied after remission there was increase of the estrin excretion.—E. L. S.

Tumors of the thyroid gland. Lake, W. F., and A. J. Ayers, South. M. J. **29:** 665. 1936.

There is a report based on 60 specimens removed at operation. Three unusual cases of thyroiditis and 2 of advanced carcinoma are presented in detail.—J. C. D.

Concerning the working point of thyroxin (Über den Angriffspunkt des Thyroxins). Mansfield, G., Fr. v. Tykudy and Irene Scheff-Pfeifer, Arch. f. exper. Path. u. Pharmakol. **181:** 376. 1936.

The mode of action of thyroxin on the metabolic rate of the normal dog and of the dog with the cervical cord sectioned was studied by these authors. The basal rate was recorded in the normal animal before and after sectioning of the cord. In animals treated with thyroxin prior to section of the cord the transection did not lower the increased basal rate. Administration of thyroxin after section of the cord produced the usual increase in the basal rate. Studies of tissue metabolism were made in vivo and in vitro under the influence of luminal narcosis. It was shown by these studies that the luminal prevented the expected increase of metabolic rate after thyroxin administration; however the failure was not due to removal of the vegetative center, but to a checking action of the tissue itself, a peripheral rather than a central action of the luminal.—R. Cleveland.

Clinical experiences in the use of determinations of blood iodine. McCullagh, E. P., and D. R. McCullagh, Arch. Int. Med. **57:** 1061. 1936.

From a study of a number of cases, it is indicated that in Cleveland the blood iodine levels of normal adults and of patients who have diseases which are not associated with the thyroid gland range from approximately 8 to 12 γ/100 cc. The blood-iodine level is very high in cases in which iodine medication has been instituted. Exercise has little influence on the blood iodine, but strenuous exertion may have a temporarily depressing effect. Under controlled conditions the level is proportionate to thyroid activity in most cases, the change in the level, however, being relatively greater in persons with hyperthyroidism than in those with hypothyroidism. Determinations of blood iodine are of value in the differential diagnosis of hyperthyroidism.—I. B.

The effect of experimental hyperthyroidism on carbohydrate metabolism. Mirsky, I. A., and R. H. Broh-Kahn, Am. J. Physiol. **117:** 6. 1936.

Data reported on 16 rabbits (8 control and 8 experimental) indicates that in the eviscerate animal previously rendered hyperthyroid by the feeding of 10 grains of desiccated thyroid daily for 6 or 7 days, the blood sugar dropped more than twice as fast as it did in control eviscerated animals. The authors conclude that in hyperthyroidism there is a marked acceleration in carbohydrate oxidation in extrahepatic tissues. The results are interrupted as supporting the hypothesis that ketosis results when there is an increased catabolism of protein and fat (gluconeogenesis) with little or no carbohydrate available for use by the liver itself.—F. A. Hitchcock.

Disturbance of vitamin C metabolism in hyperthyroidism (Skorbutische Stoffwechselstörung Hyperthyroidismus). Mosonyi, J., Klin. Wchnschr. **15:** 24. 1936.

The production of experimental hyperthyroidism in guinea pigs by thyroxin injection or thyroid feeding, resulted in a diminished vitamin C content of the liver and adrenals. Thyroidectomy in 2 rats resulted in an increased vitamin C content of the adrenals. These results are interpreted as indicating an antagonism between the thyroid secretion and the vitamin.—M. H. F.

Contribution to the question of the blood iodin level (Beitrag zur Frage des Blutjodspiegels). Pfeiffer, G., W. Mobius and K. Pohl, Arch. f. exper. Path. u. Pharmakol. **181:** 444. 1936.

The authors discussed the methods of iodin determination as used by Fellenberg and by Leipert. The technique employed by Pfeiffer involving the use of chromosulfuric acid, chromic acid and arsenious acid is described. The authors conclude from their work on determinations of blood iodin that a consistent parallelism between the blood iodin level and the degree of thyroid disorder does not exist.—R. Cleveland.

Myxedema following the removal of an aberrant thyroid tumor. Probstein, J. G., and H. Agress, New England J. Med. **214:** 1191. 1936.

A girl of 17 developed complete myxedema following removal of a right suprahyoid aberrant thyroid. Her recovery was complete following replacement therapy.—J. M. L.

Studies on the relation of diet to goiter. III. Further observations on a goitrogenic diet. Remington, R. E., and H. Levine, J. Nutrition **11:** 343. 1936.

In continuation of previously published studies it has been found that in rats on an iodine deficient ration, definite correlations exist between size and iodine content, and size and dry matter content of thyroid glands. The enlargement becomes great enough to be significant when the iodine content of the gland is below 0.03%, dry basis, corresponding to a daily intake of 0.4 γ per rat. Variations in degree of enlargement within the critical range are ascribed to uncontrollable sources of iodine, such as laboratory dust or air, and it is recommended that in studies of low iodine goiter, results should be evaluated in comparison with simultaneous control animals. The addition of iodine to the breeding ration during the first 4 weeks of life does not prevent thyroid enlargement when the animals are kept for 5 weeks on an iodine deficient ration. In contrast to the observations of Thompson and Hellwig, goiter has been produced on low iodine rations with varying calcium content, varying Ca: P ratio, and varying acid: base ratio, the single criterion as to degree of enlargement being the iodine content of the gland. Although the iodine-deficient diet used is also rachitogenic, rickets can be produced without goiter, and goiter without rickets, depending on whether iodine or vitamin D is added to the ration.—Authors' Abst., Wistar Bib. Service.

Studies on the relation of diet to goiter. IV. The antigoitrogenic value of some food. Remington, R. E., E. J. Coulson, and H. Levine, J. Nutrition **12:** 27. 1936.

Employing as criteria of low iodine thyroid hyperplasia in the rat, the fresh weight, iodine content, and dry matter content of the thyroid gland, as compared with previously published reference curves (J. Nutrition, 1936, vol. 11, p. 343), the response to adequate and inadequate intakes of iodine in the forms carried by some natural foods has been determined by a standardized technic. The foods studied were dried oysters, dried haddock, dried milk of high and low iodine content, and Irish moss. All of these except Irish moss were able to prevent goiter to an extent that is proportional to the amount of iodine carried by them, and to approximately the same extent as potassium iodide. The results with Irish moss were about one-half that indicated by its iodine content. Comparison of the results on dried milk of high and low natural iodine content indicates that the thyroid response is due to the iodine, and not to the improvement in protein quality, mineral or vitamin supply, when it was added to a goiter-producing diet in amounts as high as 20%.—Authors' Abst., Wistar Bib. Service.

Functional auto- and homoplastic thyroid grafts in the rat. Salmon, T. N., and A. E. Severinghaus, Proc. Soc. Exper. Biol. & Med. **34:** 251. 1936.

Complete success with autotransplants was obtained when the gland was transplanted subcutaneously over the ribs at the age of 1-7 days. Success was also obtained with homotransplants between day-old litter mates, with transplants of glands from day-old rats into recipients 16 to 92 days of age, and with reciprocal transplants between litter mates of 2

weeks of age. The sucess was affirmed by the growth and appearance of the recipients, by the histological structure of the transplants, and of the pituitary (absence of typical thyroidectomy change).—M. H. F.

Effect of total thyroidectomy in man. Schnitker, M. T., L. H. Van Raalte and E. C. Cutler, Arch. Int. Med. **57:** 857. 1936.

This contribution is a survey of the literature, the presentation of laboratory studies and observations of clinical effects of total thyroidectomy in 39 cases of cardiac disease. The patients were divided into 3 groups: those having angina pectoris (22), those with cardiac valvular disease in failure (15) and those with diabetes mellitus (2). Each patient was studied preoperatively as well as postoperatively, during myxedema and after the administration of thyroid substance. The basal metabolic rate declined after total thyroidectomy, reaching an average of 22.8% in 10 weeks after operation in the group with angina pectoris. The decline in the group with cardiac failure occurred in 9 weeks, with an average of 27%. Administration of 0.015 gm. of thyroid substance daily raised the level toward normal, usually in from 3 to 4 weeks. The cholesterol content of the blood rose after total thyroidectomy, reaching an average level of 404 mg. % in the group with angina pectoris in the state of myxedema. The group with cardiac failure showed an average increase to 315 mg. %. The values declined following thyroid medication. Total thyroidectomy was followed by a striking decrease in the vital capacity in 50% of the patients with angina pectoris, the capacity returning to normal within 1 week. Of the group with cardiac failure, 60% showed an average increase of 24% in vital capacity when in a myxedematous state; the remaining 40% showed no appreciable change. A slowing of the volume of blood flow to 43% as compared with normal flow occurred in the group with angina pectoris in a myxedematous state. The group with cardiac failure showed a similar change, but the slowed velocity in persons with decompensation masked that associated with myxedema. The administration of thyroid substance caused an increased velocity of blood flow, which was superseded by a fall if decompensation ensued. The Ca and P content of the blood fluctuated considerably but remained within normal limits. Tetany did not follow removal of the parathyroid glands in several cases. The value for total blood protein was lowered to the lower limits of normal in cases of induced myxedema. The value was not altered by thyroid medication. The albumin-globulin ratio remained unaltered. The blood K varied in both directions but remained relatively constant following total thyroidectomy. Persons with normal thyroid glands, as well as those with induced myxedema, showed a great variation in the blood iodin content (16 patients). Immediately following total thyroidectomy there was an increase in I; the value reached a peak in from 3 to 6 days (2 patients) and then declined to normal. Two patients with mild diabetes mellitus were distinctly benefited by total thyroidectomy. In a comparison of spontaneous and induced myxedema, it was noted that induced myxedema did not cause the degree of anemia observed in the group with spontaneous myxedema. Total thyroidectomy was followed by an elevation of 10 mm. Hg. in both the systolic and the diastolic blood pressure, although there was no essential change in the pulse rate. Postoperative muscular pains in the legs followed total thyroidectomy; these were relieved by thyroid medication or exercise.—I. B.

Hyperthyroidism and gastric secretion (Basedow'sche Krankheit und Magensekretion). Schwanke, W., Klin. Wchnschr. **14:** 1849. 1935.

From single pre-operative and postoperative determinations of the fasting free acidity and the response to an alcohol-test meal, the author presents a table of the results obtained from 46 patients with hyperthyroidism, purporting to show subnormal values before operation with recovery toward the normal, in some instances, following operation.—M. H. F.

Effect of total thyroidectomy on response to injection of adrenalin. Seely, H., and E. C. Cutler, Proc. Soc. Exper. Biol. & Med. **34:** 23. 1936.

Thyroidectomy in dogs markedly decreased the skin temperature response to adrenalin in a limb deprived of sympathetic innervation.—M. H. F.

Effects of vitamin E deficiency on the thyroid gland of the rat. Singer, Eleanor, J. Physiol. **87:** 287. 1936.

Absence of vitamin E from the diet changes the normal active rat thyroid to one with much colloid and low cuboidal cells. The normal appearance was restored by E extracts or wheat germ oil, and partially by alkaline anterior pituitary extracts. Iodine administration for

4 or 10 days did not affect the histological appearance, nor did 7 days of pregnancy. The table lists 11 types of experiments on 57 rats and there are two plates with photographs of the thyroid sections.—R. L. Z.

Effect of castration on thyroid in female guinea pigs. Starr, P., and R. C. Bruner, Proc. Soc. Exper. Biol. & Med. **33:** 465. 1935.

Castration of female guinea pigs did not alter the histology of the thyroid, the response of the thyroid to thyrotropic extract, or the metabolic response to the same extract.—M. H. F.

Influence of iodine prophylaxis upon the thyroid gland of the new-born. (Über den Einfluss der Jodprophylaxe auf die Schilddrüse des Neugeborenen). Steinmann, B., Endokrinologie **16:** 395. 1936.

The author studied autopsy records of new-born infants in the Canton of Bern, Switzerland, for a period of years before and after the introduction of iodized table salt as a preventive for simple goitre. He notes a clearly perceptible, although not great, reduction in the absolute and relative weights of the thyroid glands. It is possible that the action must be ascribed in part to the use of other iodine-containing medicaments. The percentage frequency of struma congenita in the autopsy material likewise has definitely decreased in the last ten years. Iodine treatment also results in a histological structure of the thyroid which more nearly approaches the normal.—W. J. A.

The iodine content of the thyroid following chronic fluoride administration. Stormont, R. T., F. L. Kozelka, and M. H. Seevers, J. Pharmacol. & Exper. Therap. **57:** 143. 1936.

Sodium fluoride administration to rabbits caused a 20% increase in adrenal weight and a negligible increase in thyroid iodine.—E. L.

Progressive exophthalmos following thyroidectomy. Thomas, H. M., Jr., and A. C. Woods, Bull. Johns Hopkins Hosp. **59:** 99. 1936.

A study was made of 15 patients in whom progressive exophthalmos developed following thyroidectomy. Eleven of the patients were males; 4 were females. In every case thyroidectomy relieved all the general symptoms of thyrotoxicosis except the exophthalmos, which was of low degree and receded somewhat after thyroidectomy, only to recur and gradually grow worse. In several cases a characteristic lesion of the extra-ocular muscles was found. No tenable theory is at present available to account for the exophthalmos of hyperthyroidism.—A. G.

Basedow as an occupational disease. (Basedow als Gewerbekrankheit). Vannotti, A., Deutsches Arch. f. klin. Med. **178:** 610. 1936.

The author describes 2 typical cases of Basedow's disease following intoxication with carbon monoxide in 1 case and lead in the other. The author believes that a careful investigation of cases of industrial poisoning will disclose a more frequent coincidence of hyperthyroid complications in toxic conditions leading to localized or diffuse pathological involvement of the central nervous system and to changes in the peripheral oxidation capacity.—E. L.

The treatment of simple goiter. Webster, B., Internat. Clin. **2:** 168 (Series 46). 1936.

The author recommends the administration of iodine in the diffuse form of simple goiter. In the nodular form, subtotal thyroidectomy is advised if iodine treatment fails of its objective. Following subtotal thyroidectomy iodine is administered as a prophylactic against recurrence. The problem of the treatment of simple goiter is primarily one of prevention. Failing that, our attempts should be directed toward obtaining a return to as near a normal histological and physiological state of the thyroid as possible. Beyond that comes the management of complications by surgical methods.—I. B.

Morphologic changes in the heart in experimental myxedema. Webster, B., and C. Cooke, Arch. Int. Med. **58:** 249. 1936.

Myxedema was produced in adult rabbits by total removal of the thyroid gland. In the cases of more severe involvement this was accompanied by pericardial and peritoneal effusions. The heart muscle of these myxedematous animals had an average fluid content of 81.9%, as compared with 75.6% in a control series of normal animals. On microscopic examination the myxedematous heart muscle showed marked degenerative changes, characterized by a decrease in the number of fibers, edema, and a disappearance of the perinuclear sarcoplasm. Myxedema is apparently capable of producing serious myocardial damage in the adult rabbit.—I. B.